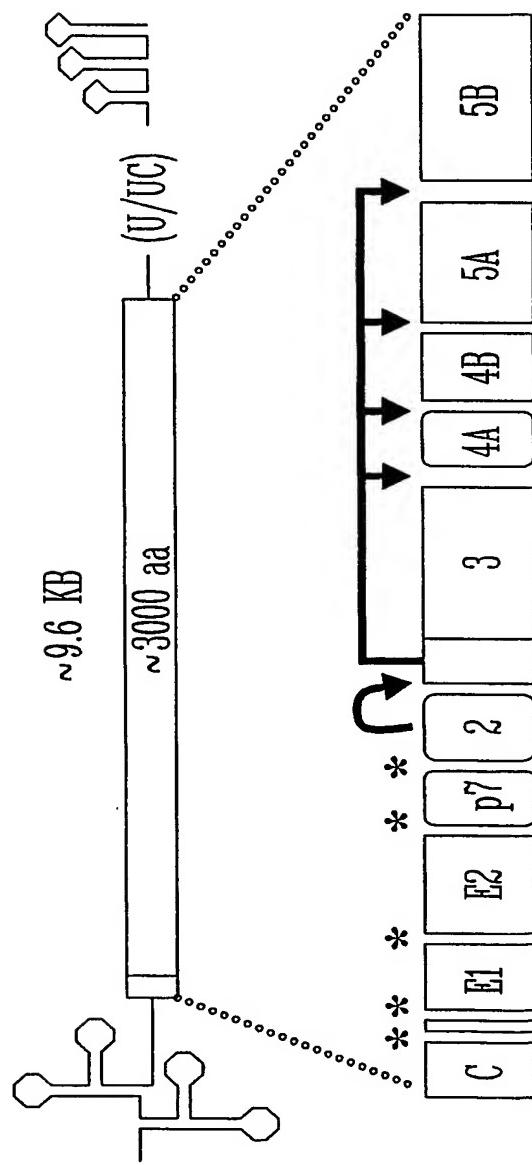


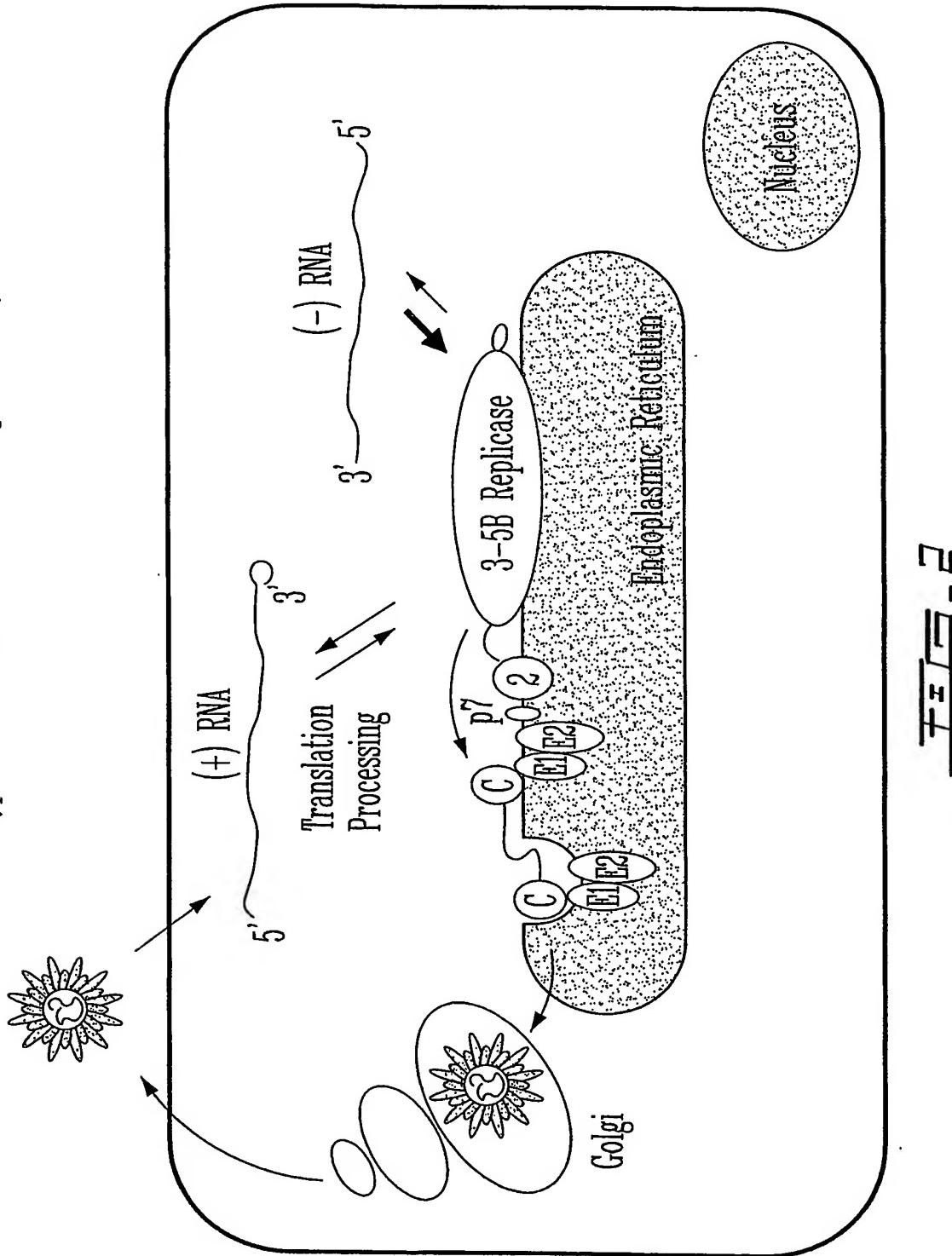
1 / 72

Hepatitis C virus (HCV) genome organization.

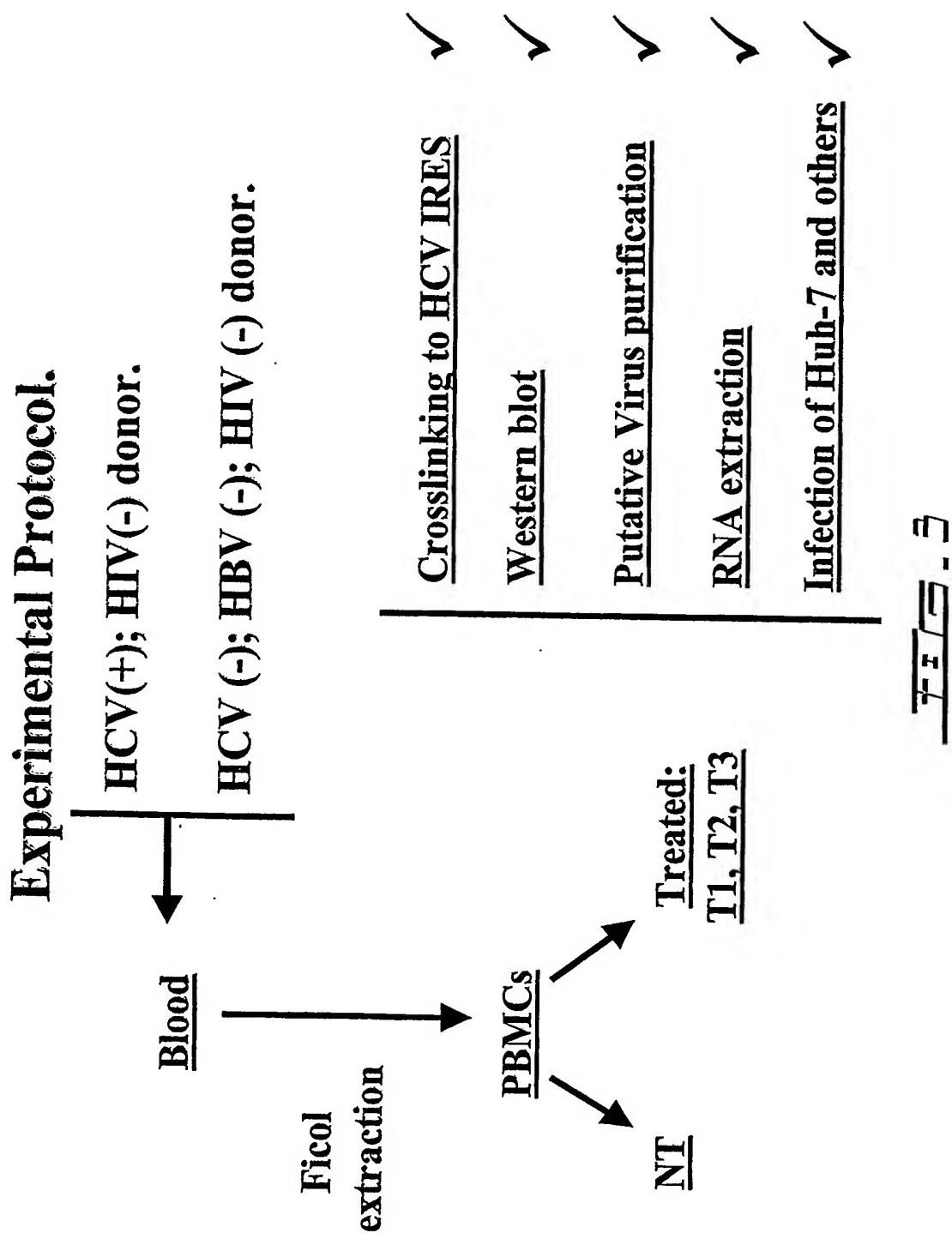


2 / 72

Hypothetical model of the HCV replication cycle

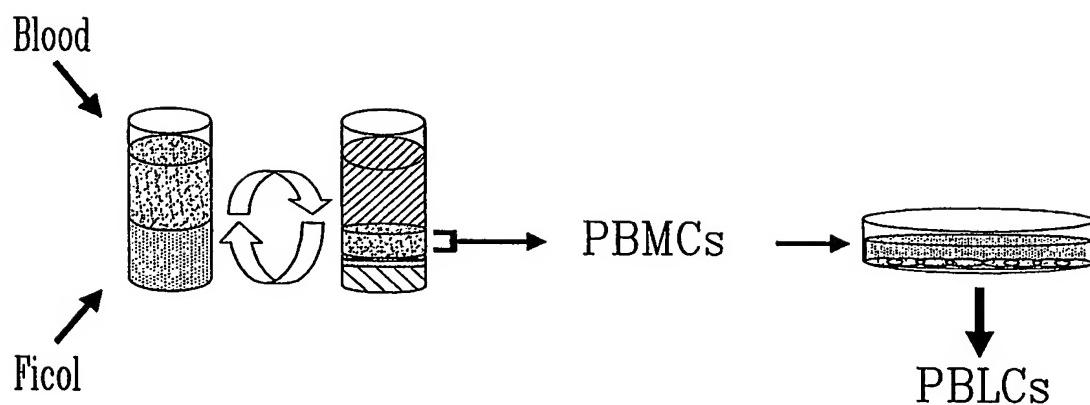


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PBMC and PBLC purification from blood samples.

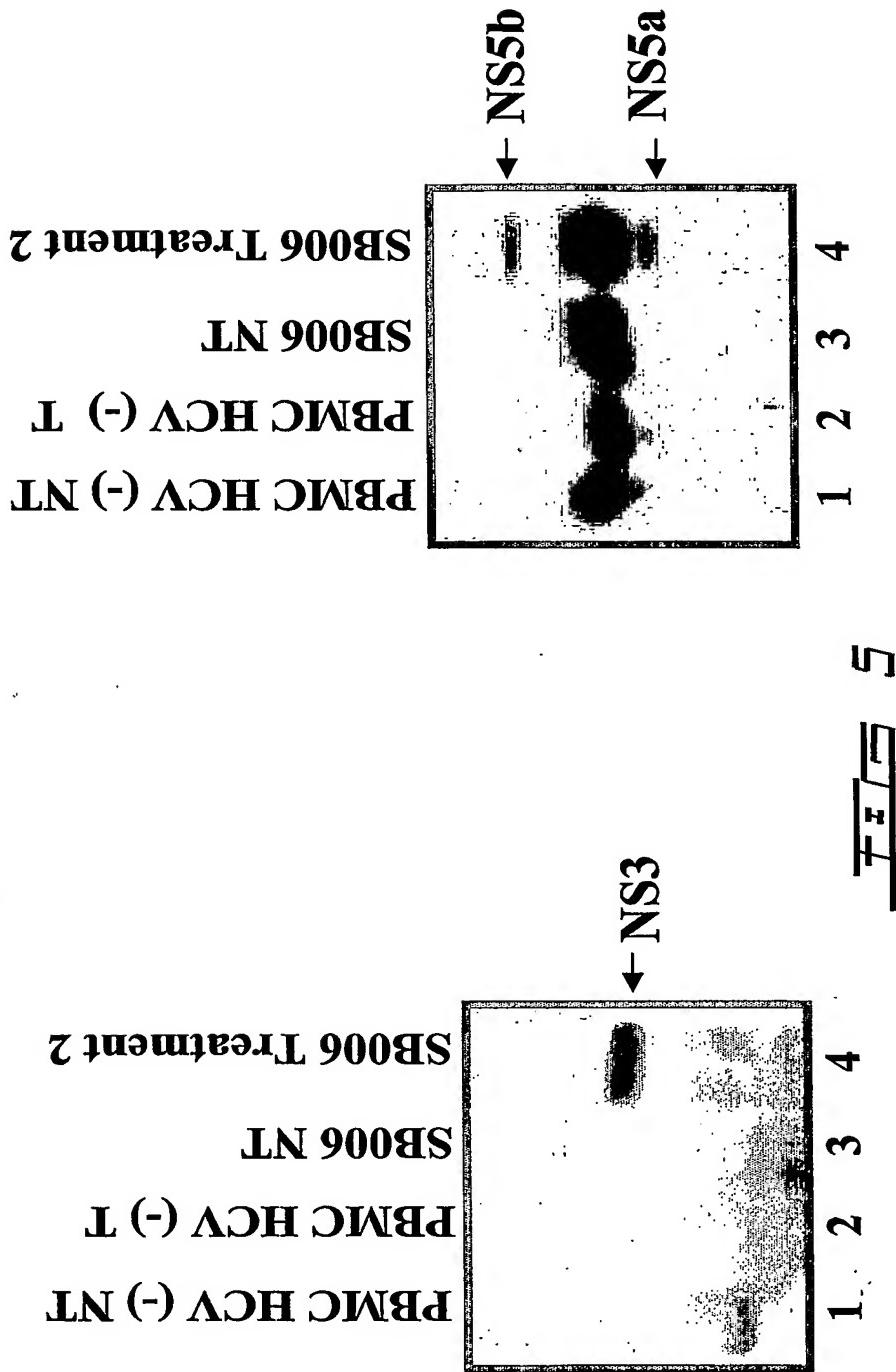


T E S T - 4

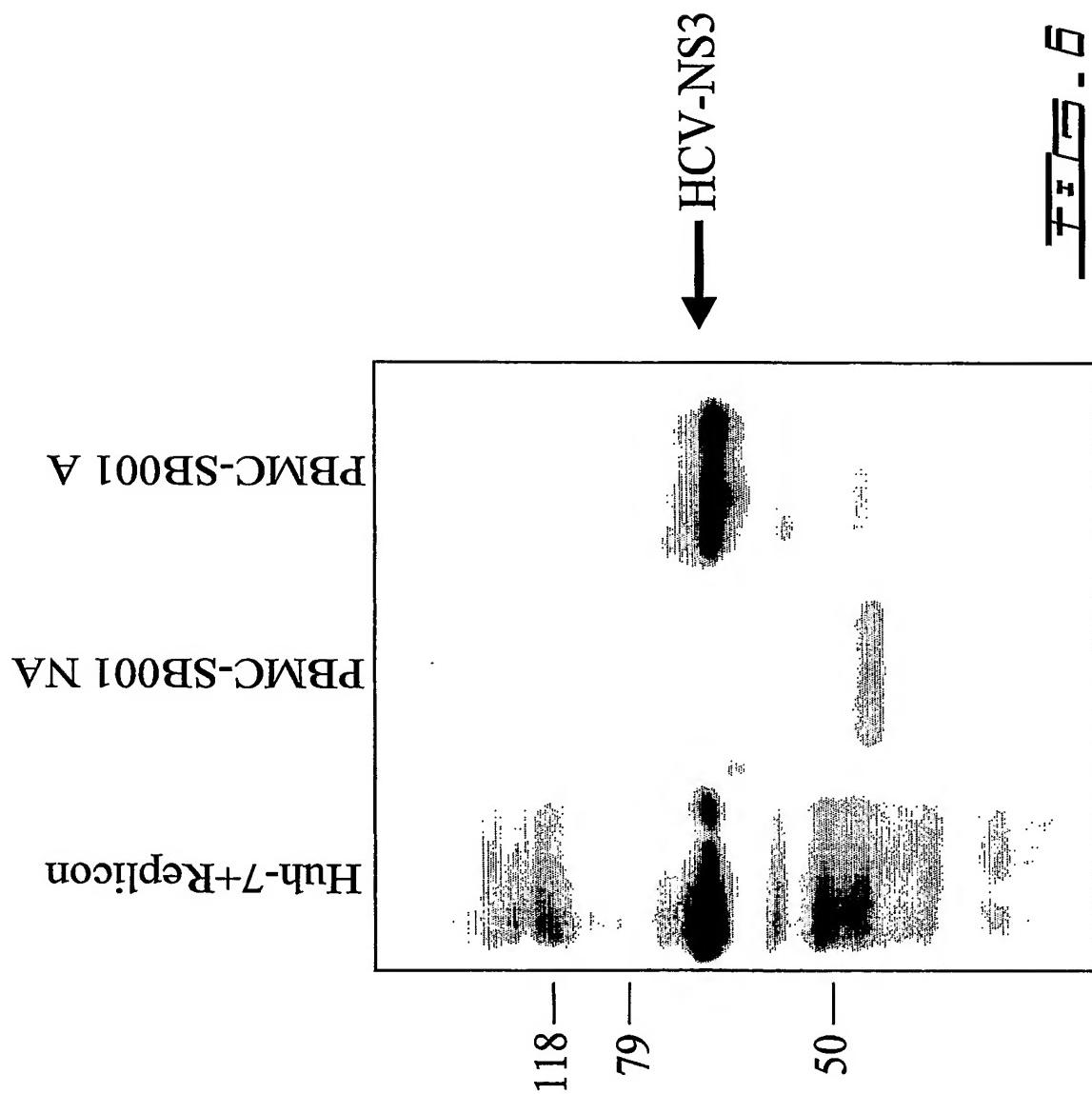
5/72

## Detection of HCV NS3 and NS5 proteins in cell extracts from Treated PBMC from an HCV (+) patient.

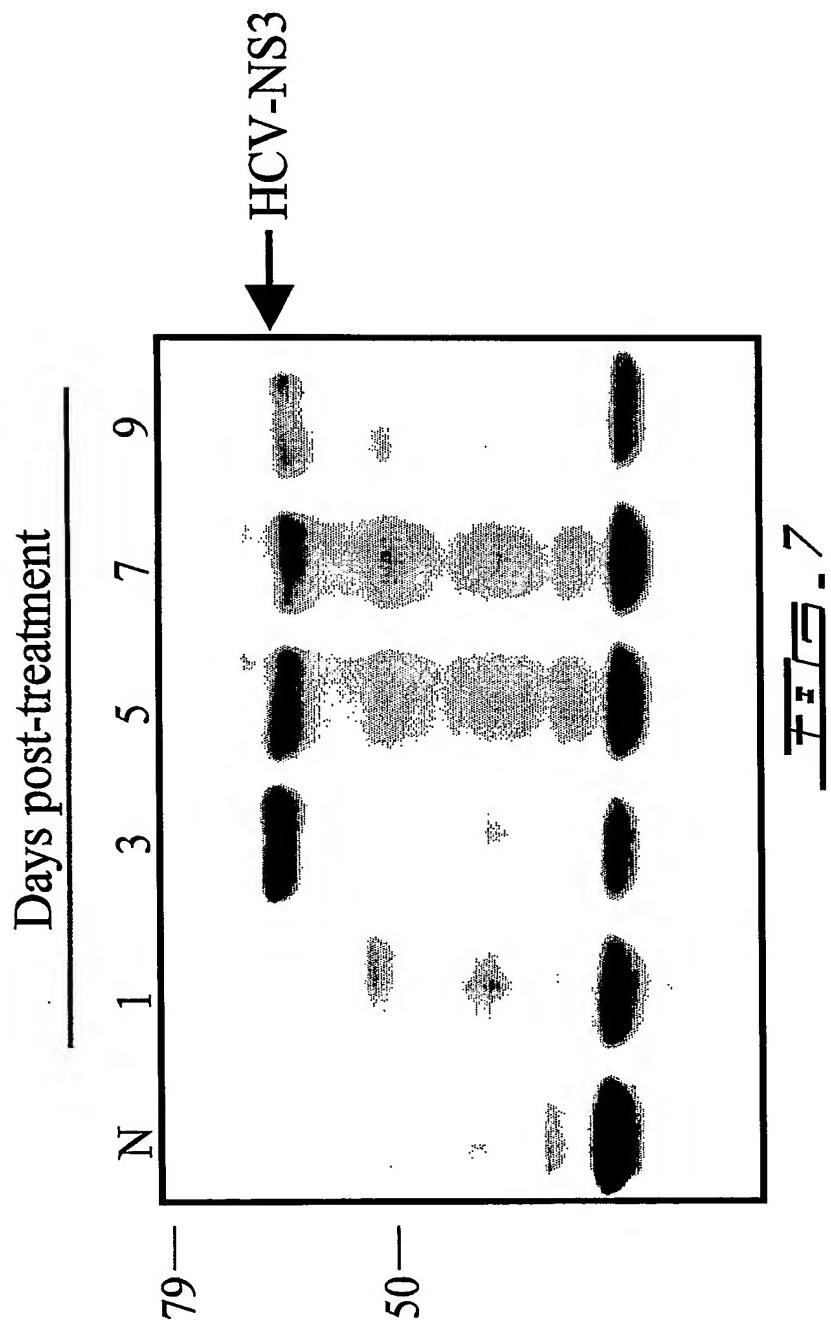
[Boeringer anti-NS3 polyclonal antibody]



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# Time course of HCV-NS3 detection: PBMcs From patient MLL-001



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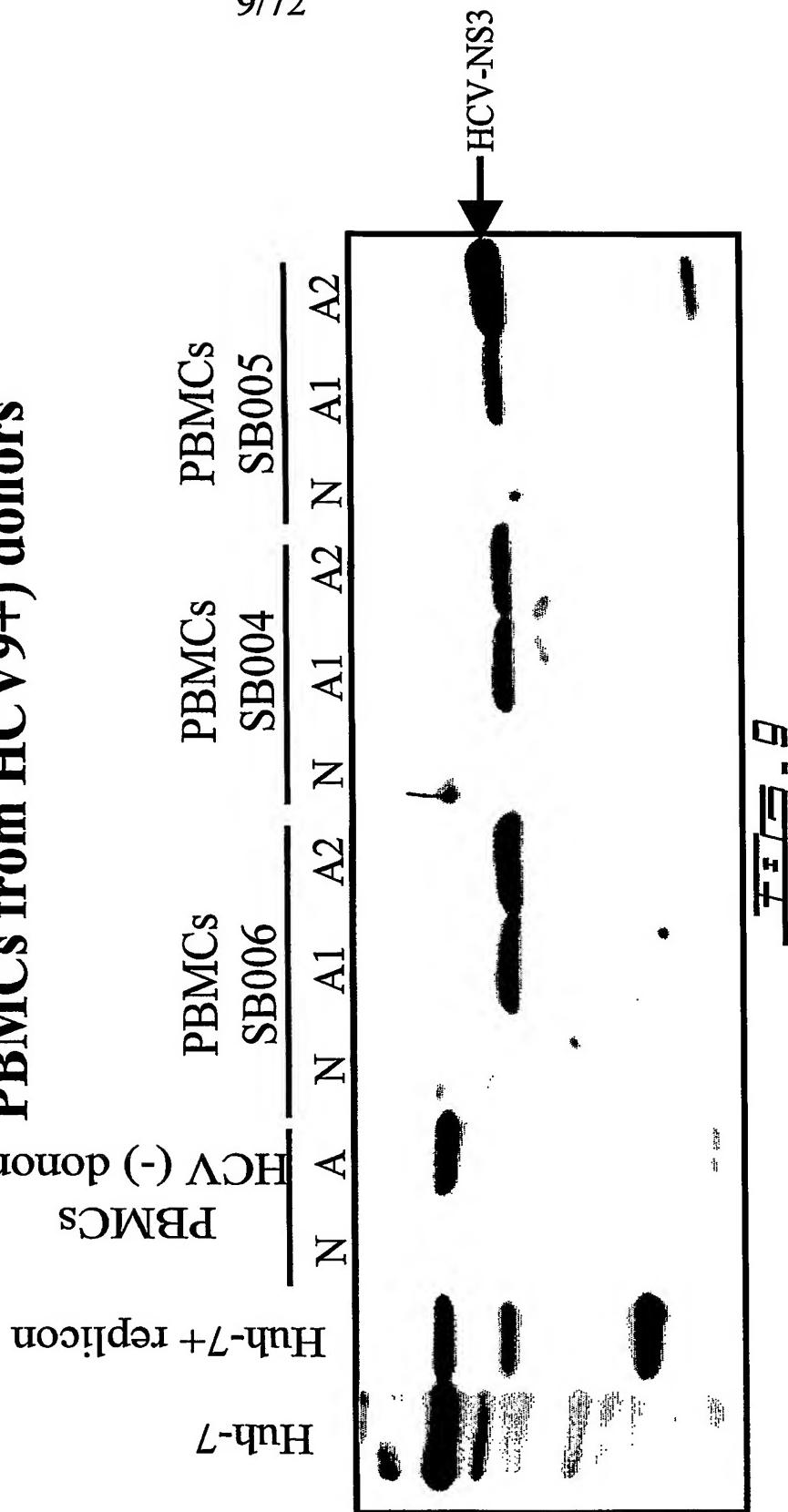
# Time course HCV-NS3 detection: PBMCS from patient MLL-002

The figure displays a Western blot analysis of HCV-NS3 protein expression. The top horizontal axis is labeled "Days post-treatment" with values 1, 3, 5, 7, and 9. The left vertical axis is labeled "N" (Normal control). Two lanes represent patient samples: lane 79 at the bottom and lane 50 at the top. A large arrow points downwards from the 9-day position towards the lanes for patient 50. Each lane contains five horizontal bands corresponding to the treatment days. In patient 79, bands are visible at days 1, 3, 5, 7, and 9. In patient 50, bands are visible at days 1, 3, 5, and 7, with a faint band appearing at day 9.

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Detection of HCV-NS3 protein in treated (N3)  
PBMCS from HCV9+) donors



Detection of virus like particles by scanning electron microscopy

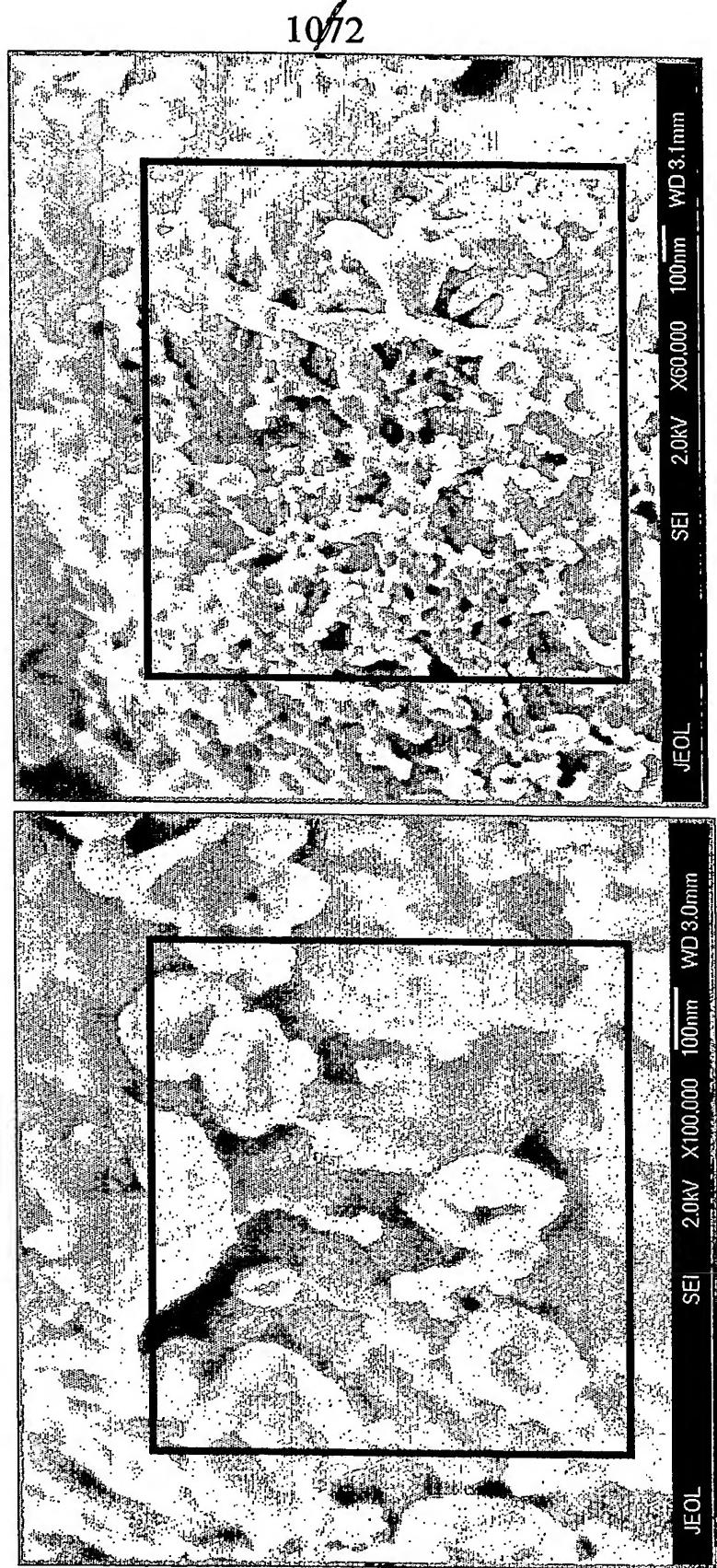
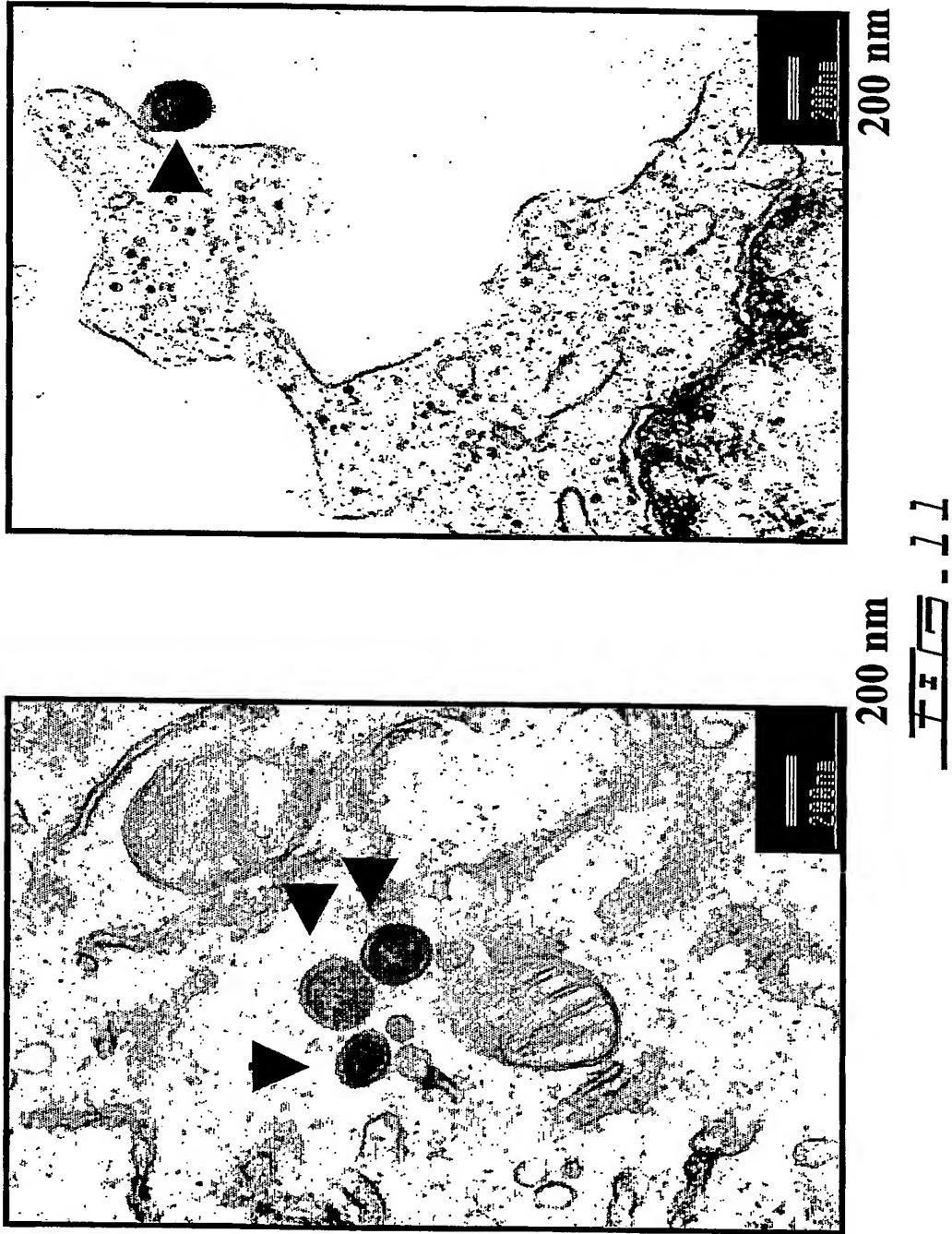


FIG - 10

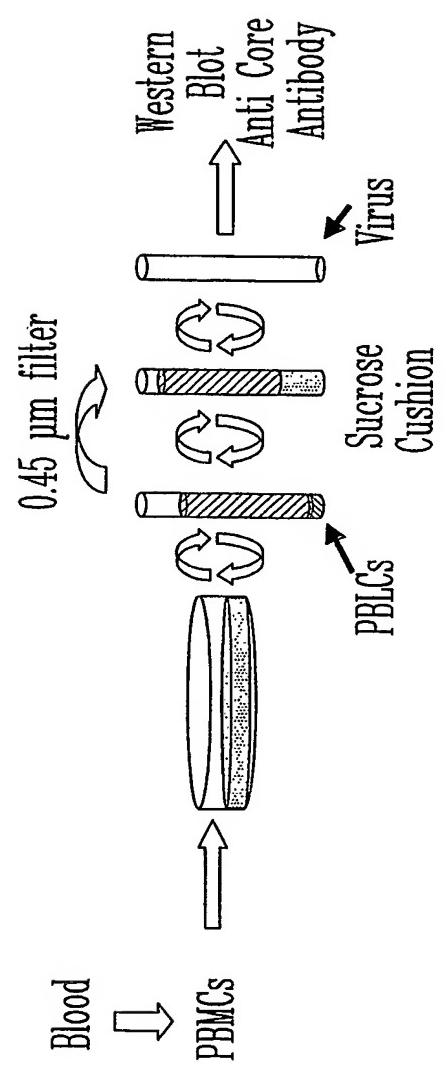
11/72

Electron microscopy of Activated PBLCs;  
Detection of virus like particles



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## Virus partial purification.

~~FIGURE - 1 E~~

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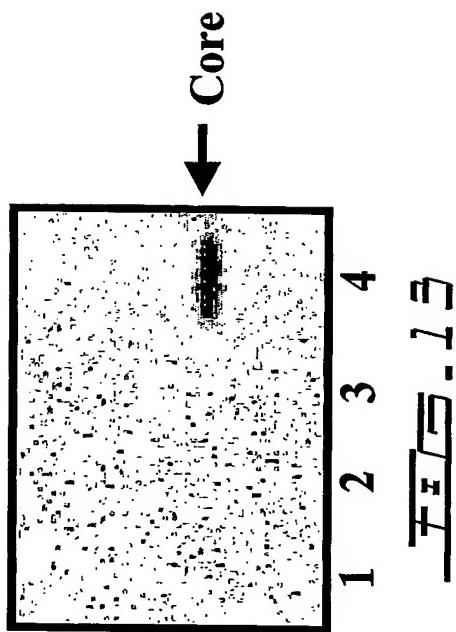
**Detection of HCV Core protein in supernatant of treated**

**PBMC from an HCV (+) patient.**

**[Maine biotechnology anti-Core monoclonal antibody]**

PBMC HCV (-) NT  
PBMC HCV (-) T

SB006 NT  
SB006 T



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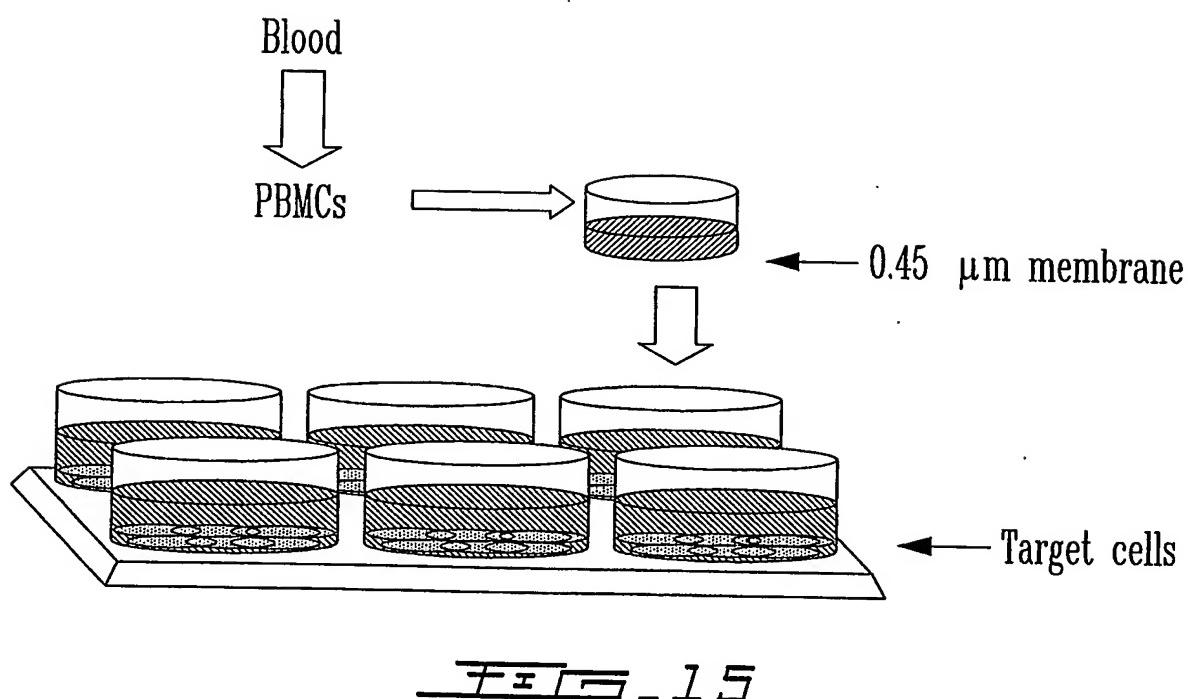
## RNA Quantification I (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant
<u>After 4 days</u>		
SB004 NT	2x10 <sup>3</sup>	No
SB004 T	2x10 <sup>3</sup>	Yes
<u>After 20 days</u>		
SB006 NT	1.8 x10 <sup>3</sup>	No
SB006 T	2x10 <sup>2</sup>	Yes
SB004	0.00	
SB006	0.00	

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## Infection assay; co-culture



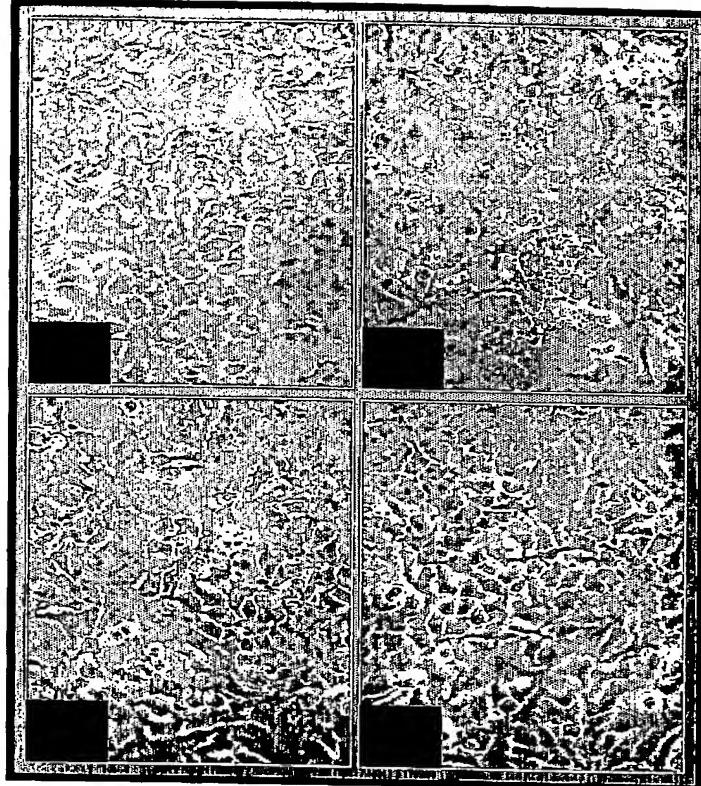
16/72

**Infection of MT-4 cells**  
**RNA Quantification II (virus copies/ng total RNA)**

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant	HCV RNA In MT-4
<u>After 10 days</u>			
SB001 NT	13	No	0.00
SB001 T	12	Yes	1600
<u>After 20 days</u>			
SB001	0.00		0.00
SB001	0.00		0.00
			<u>7575 - 16</u>

**Co-culture of Huh-7 and HCV (-) PBMCS.**

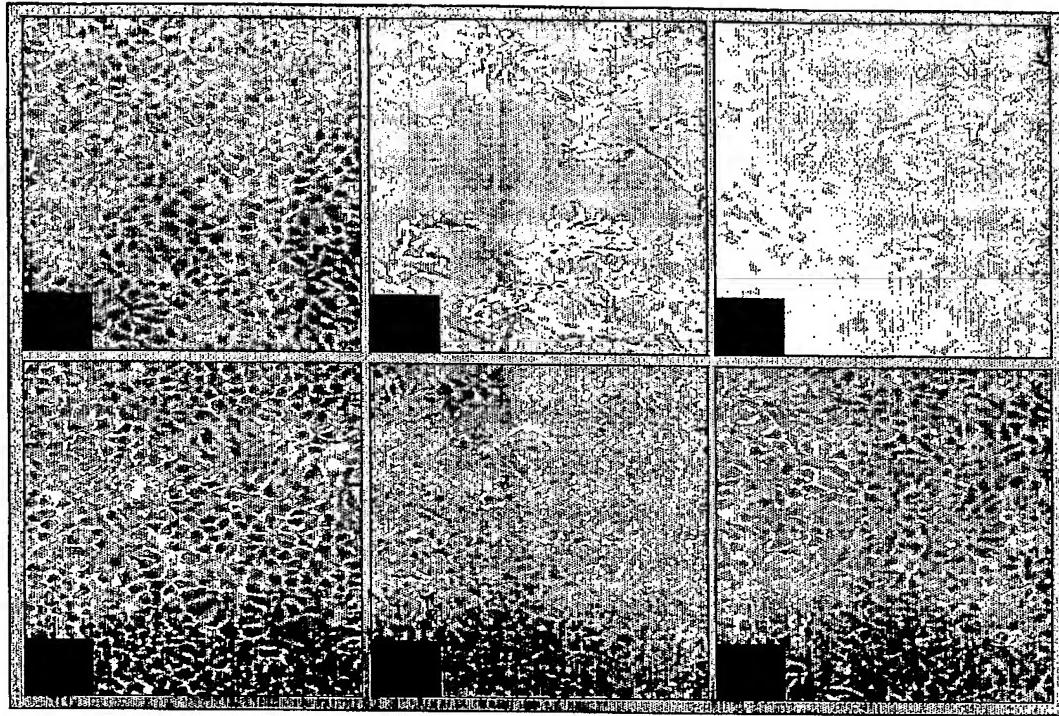
17/72



- 1- Huh-7
- 2- Huh-7 + PBMCS HCV (-) NT
- 3- Huh-7 + Treatment
- 4- Huh-7 + PBMCS HCV (-) T

FIGURE - 17

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**Co-culture of Huh-7 and HCV (+) PBMS°Cs (SB006).**

1. Huh-7
- 2-3. Huh-7 + PBMCs HCV (+) NT
4. Huh-7 + Treatment
- 5-6. Huh-7 + PBMCs HCV (+) T

7-8 - 18

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PHA Activation of PBMCS from patient SB004;  
HCV is not in T cells

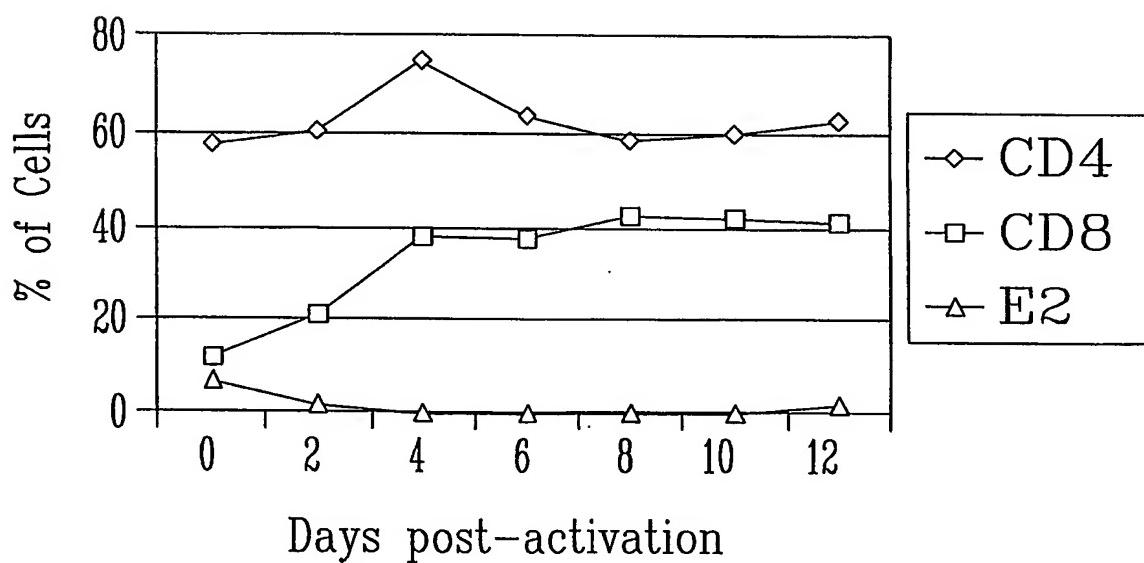
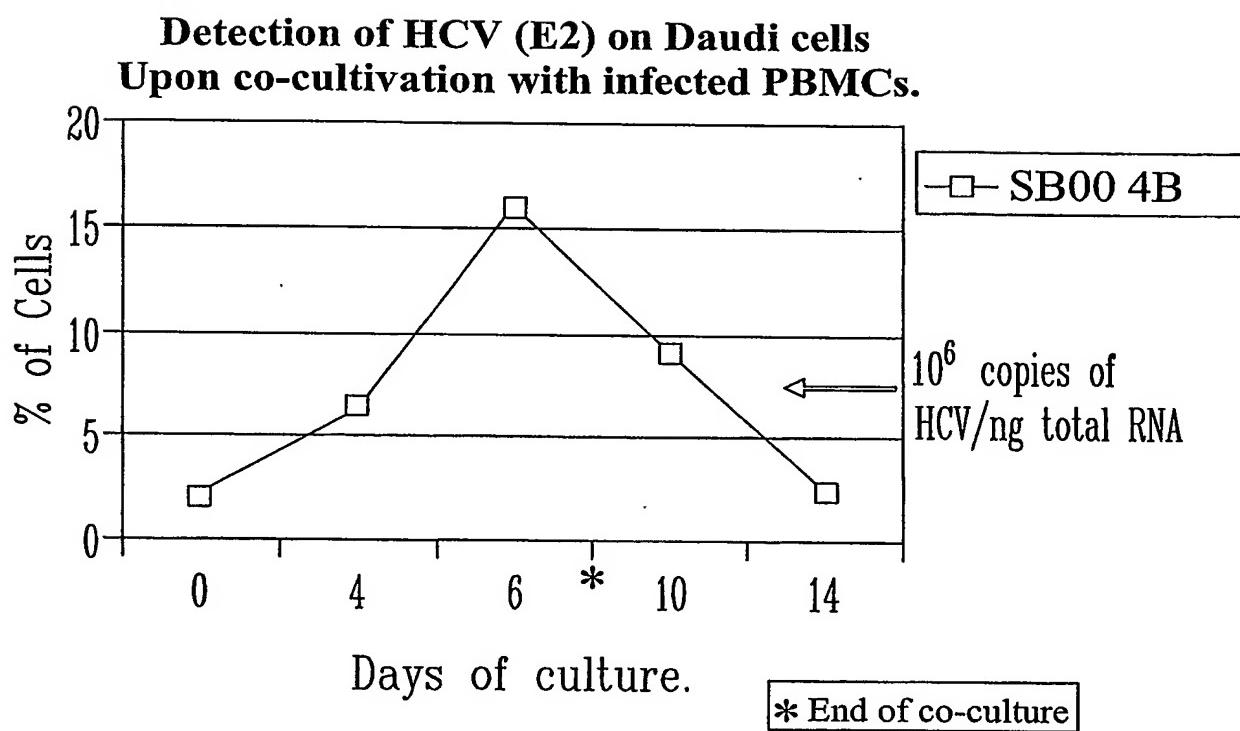


FIG-19

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FIG - 20

## Comparison of different activation treatments; PBMCs from donor MLL-010

**T cells (T1)**

**B cells (T2)**

**T+B cells**

**Treatment**

**Days**

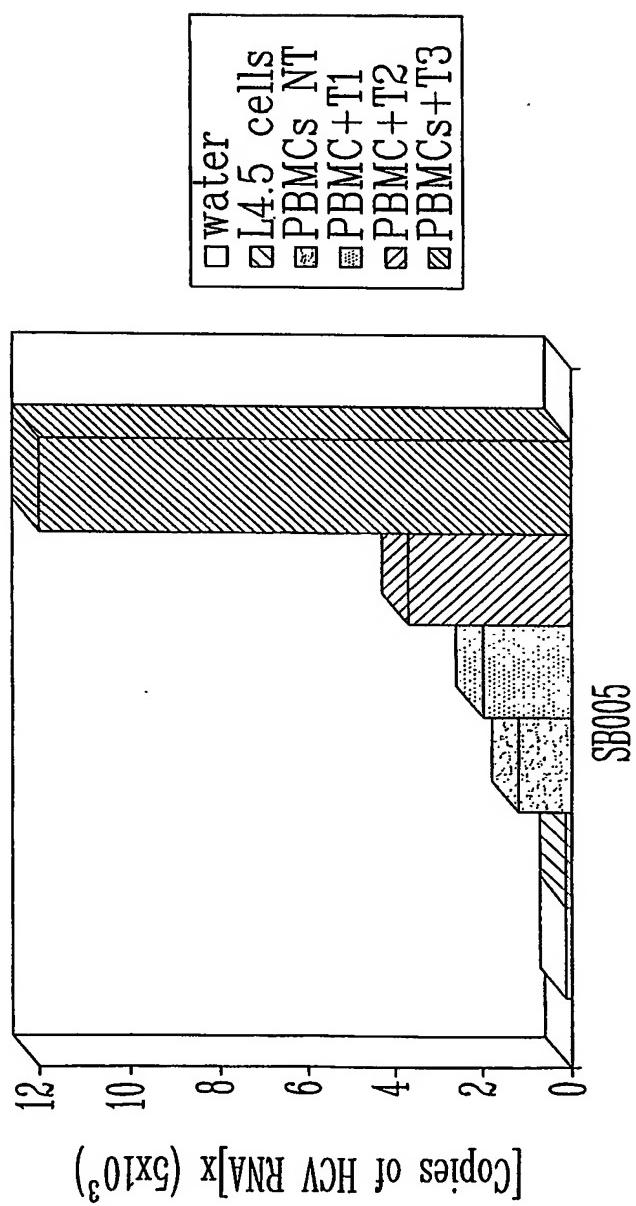
**NS3**

N	2	4	8	12	2	4	8	12	2	4
T cells (T1)	1	1	1	1	1	1	1	1	1	1
B cells (T2)	1	1	1	1	1	1	1	1	1	1
(T3)	1	1	1	1	1	1	1	1	1	1

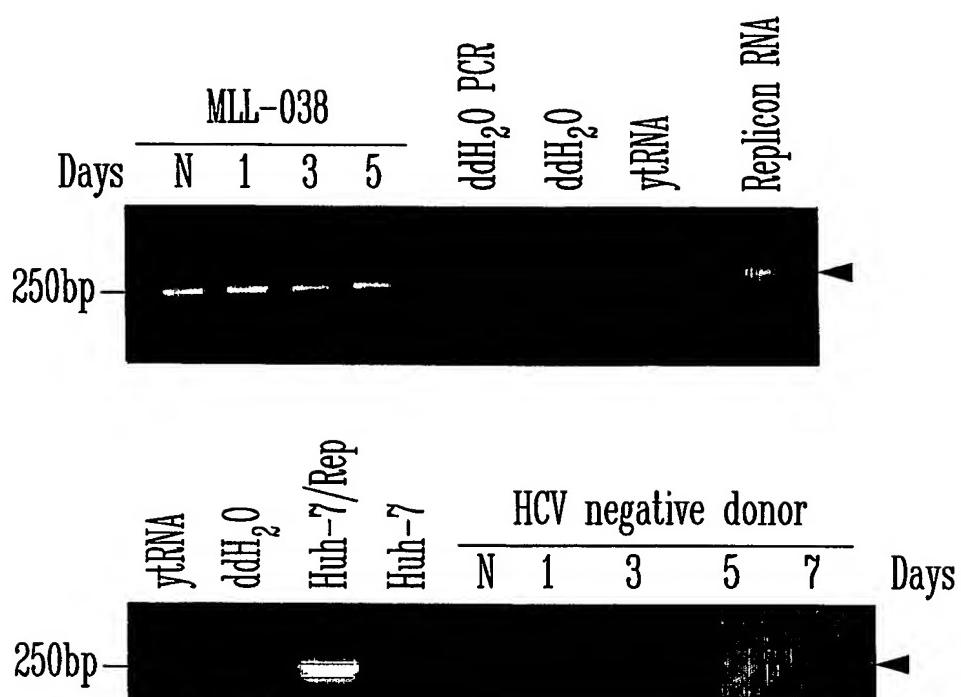
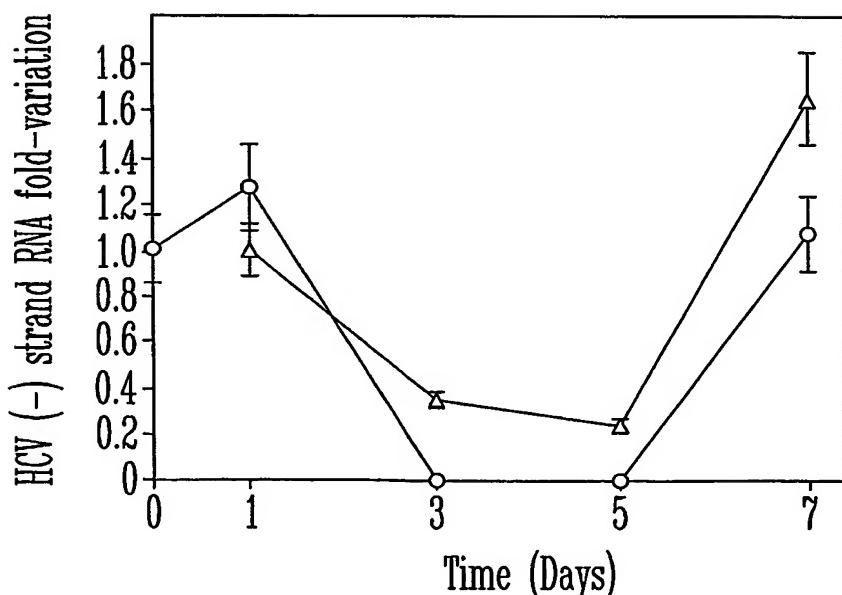
**Western Blot:** NS3

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Viral RNA in cell supernatant (Real time RT-PCR).

~~FIGURE - 22~~

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Fig. 23 AFig. 23 B

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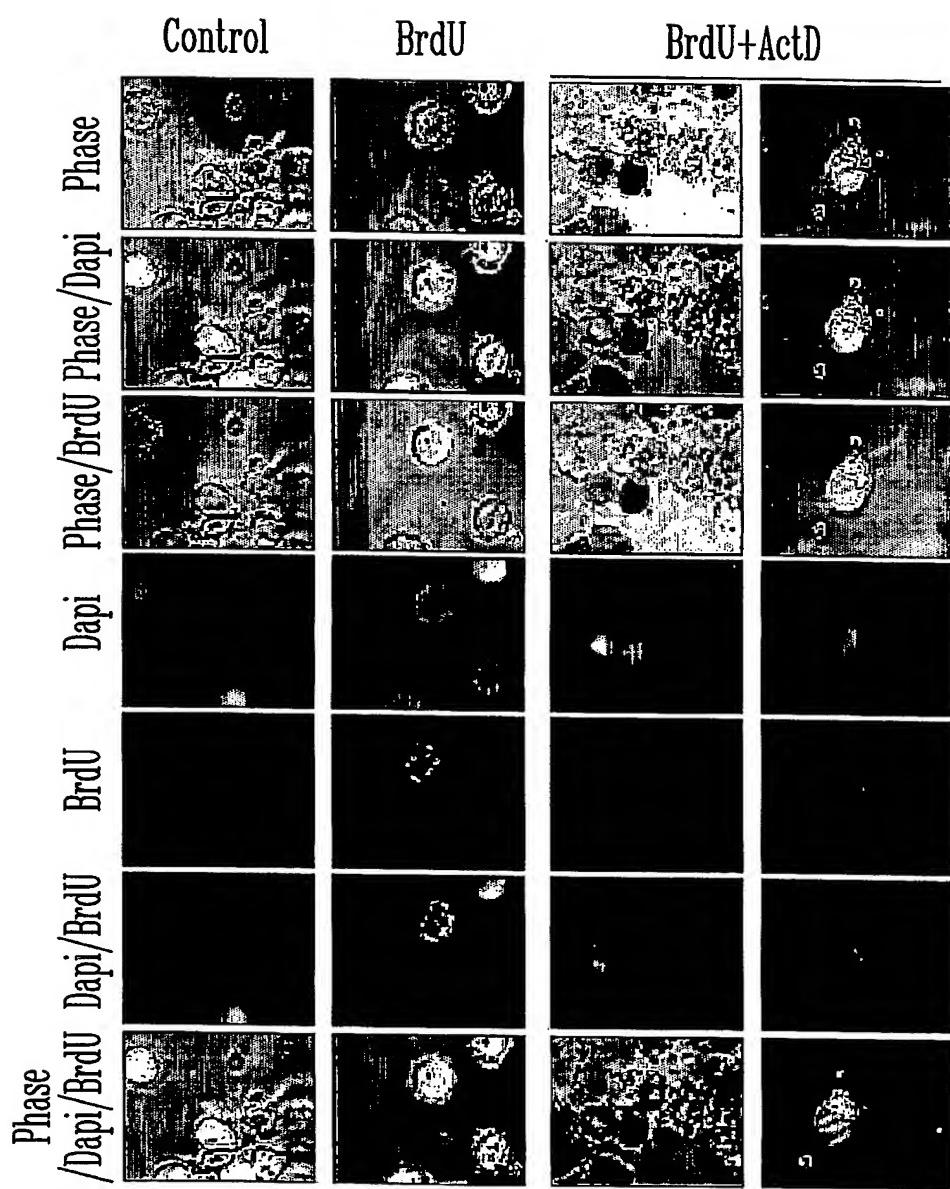


FIG. 23C

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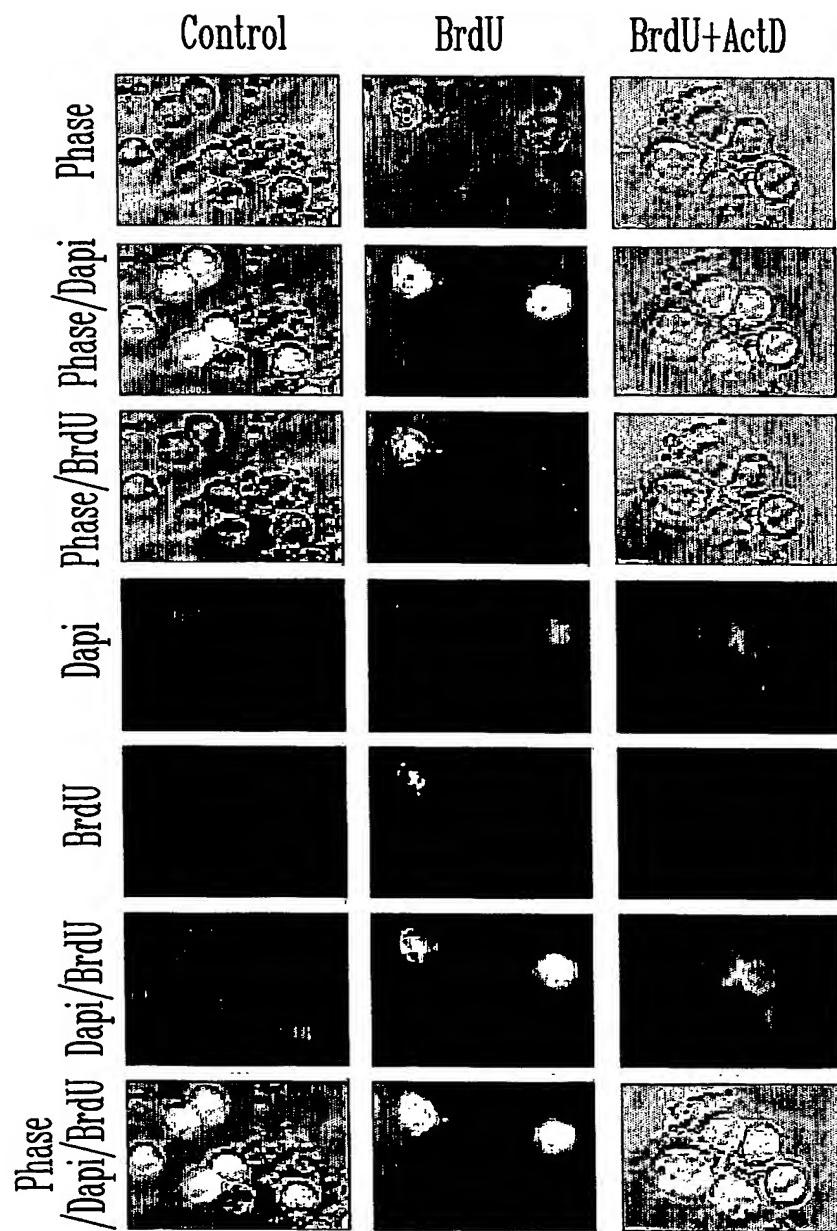
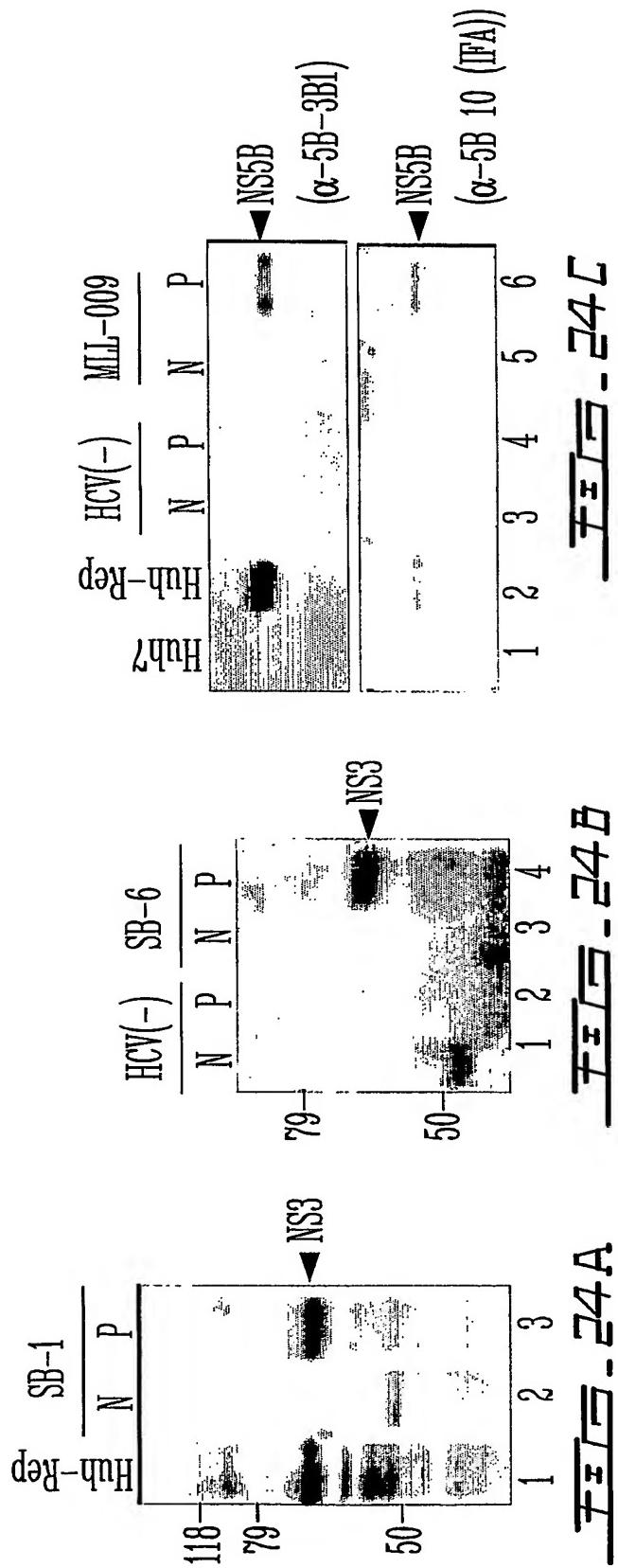
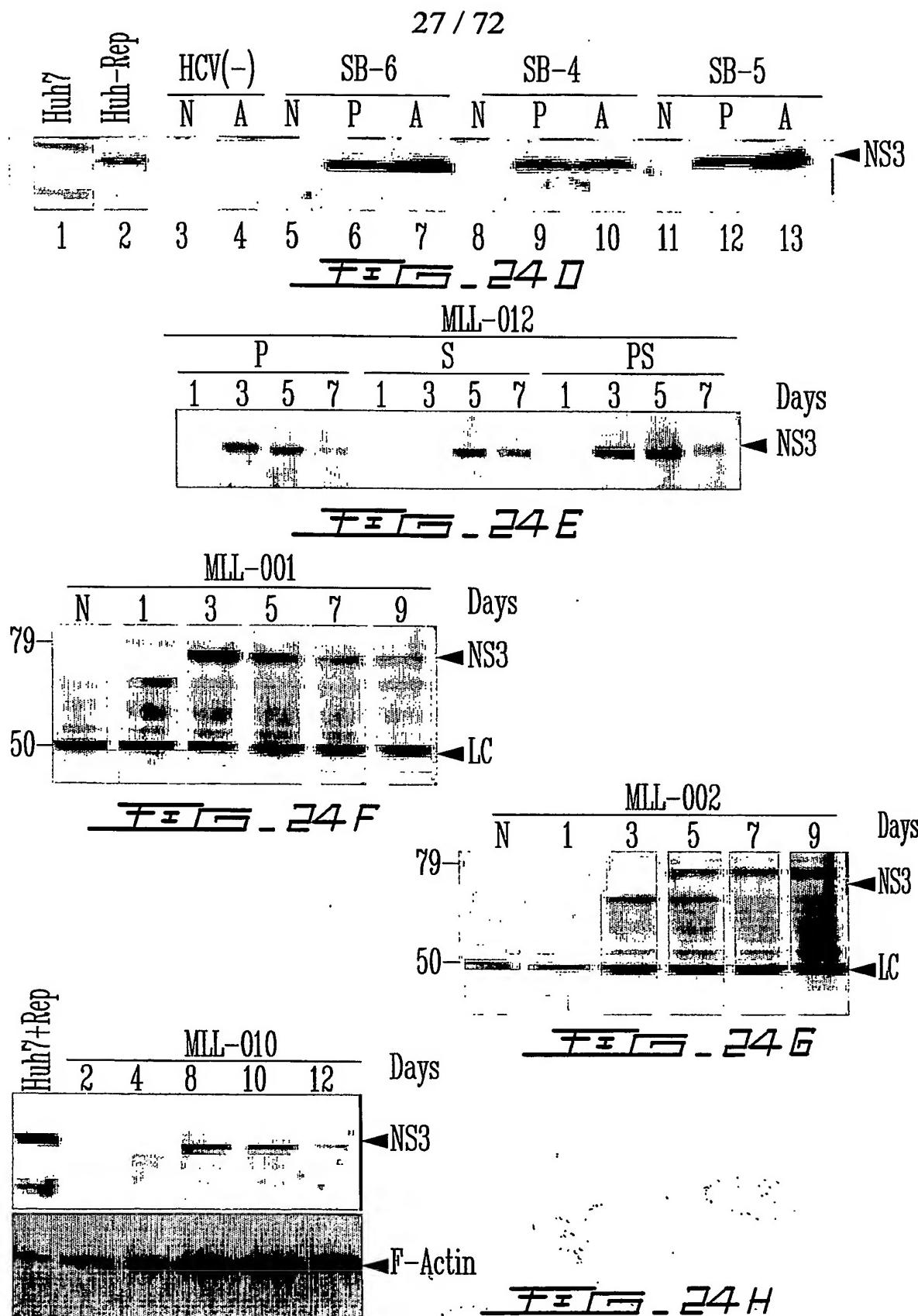


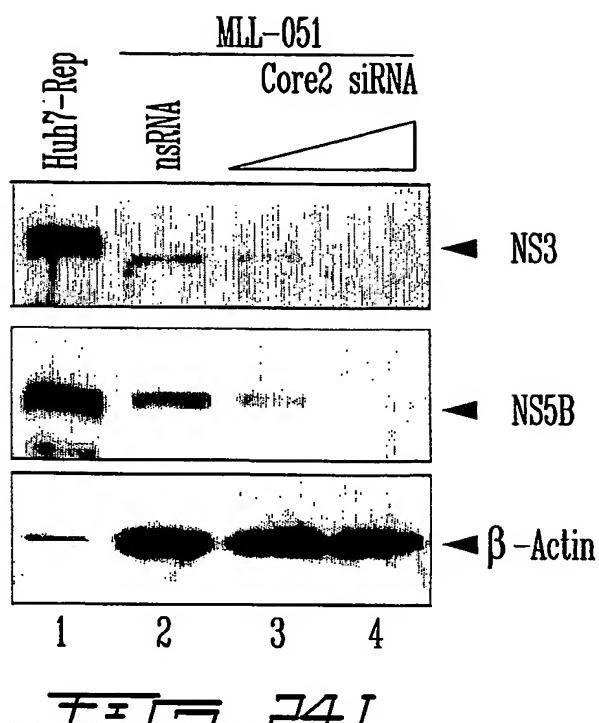
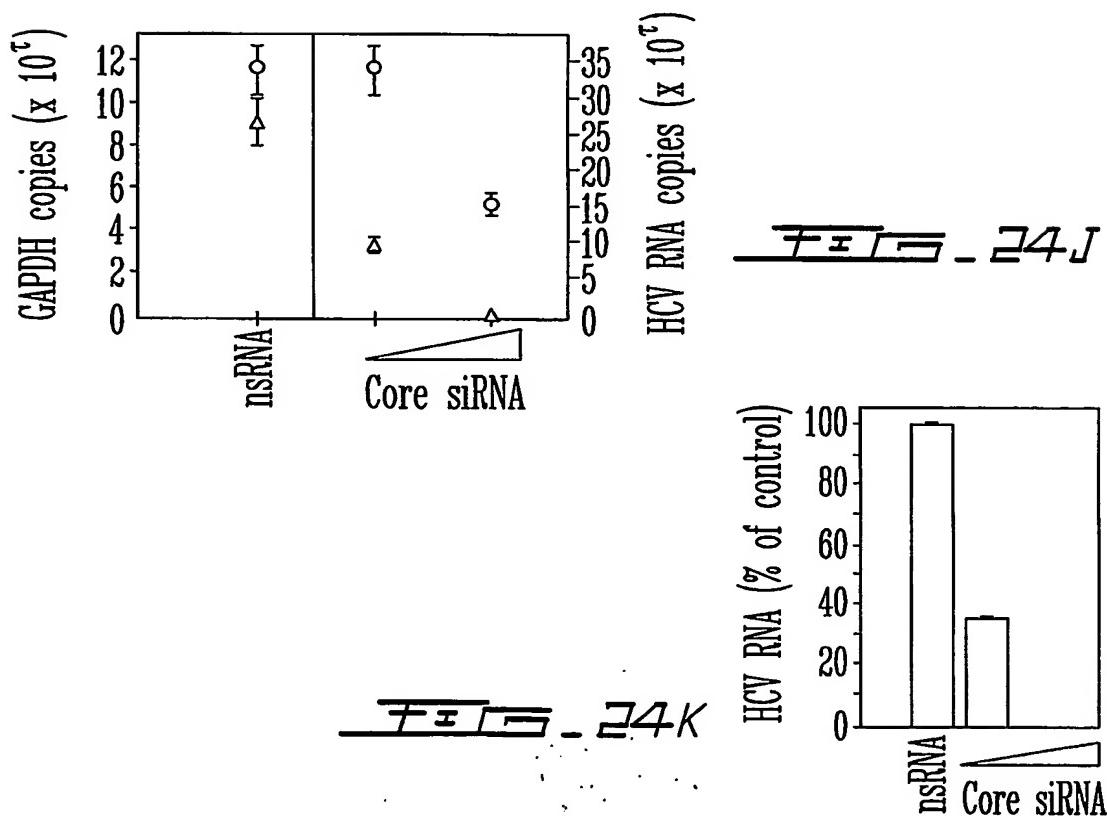
FIG - 230

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F I F - 24 I

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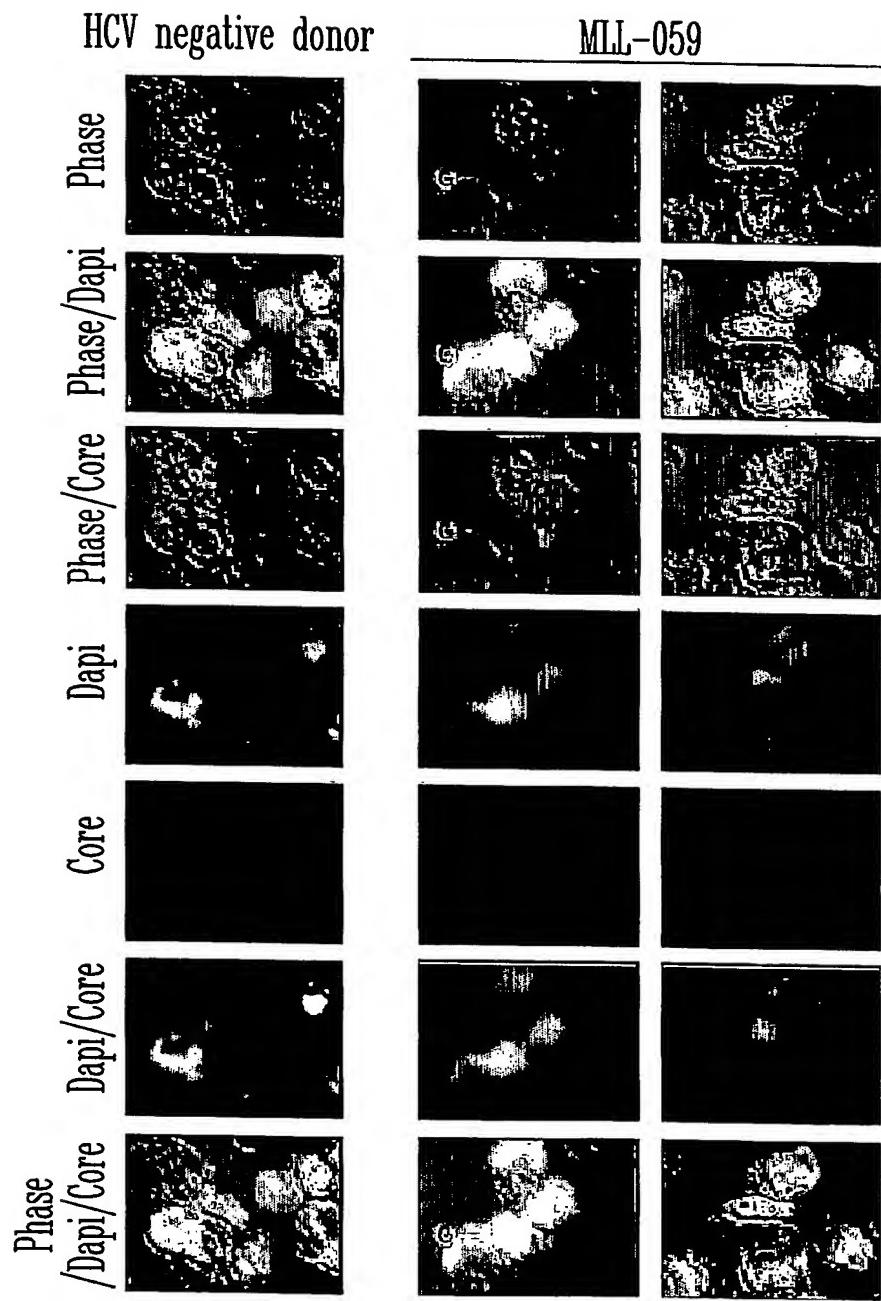
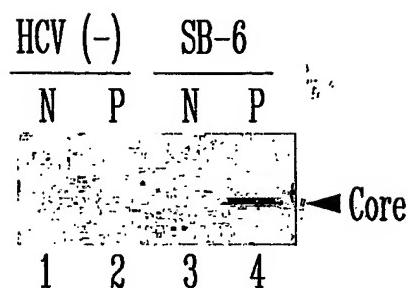
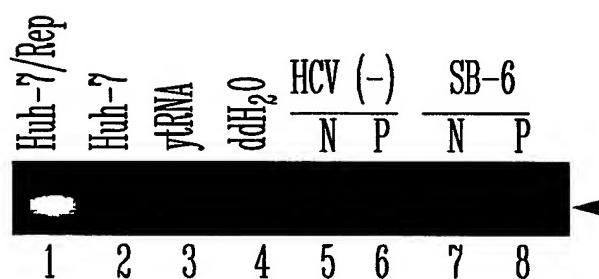
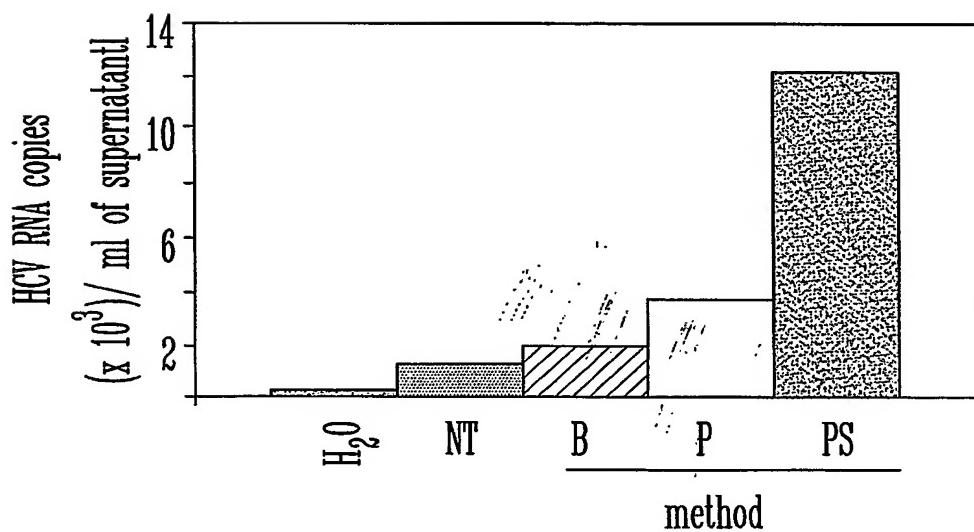
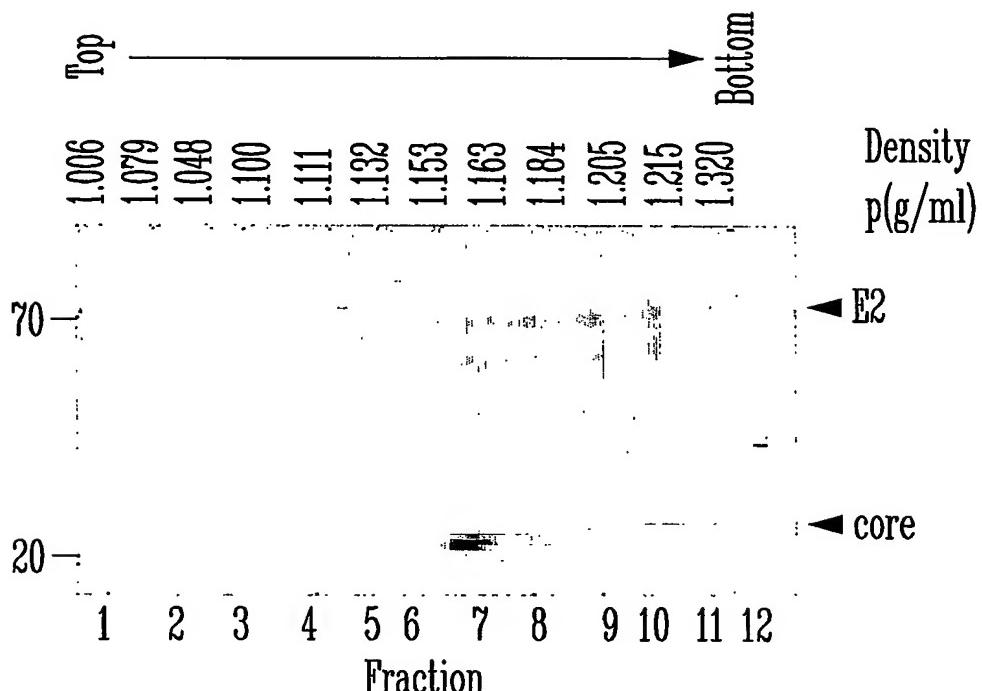
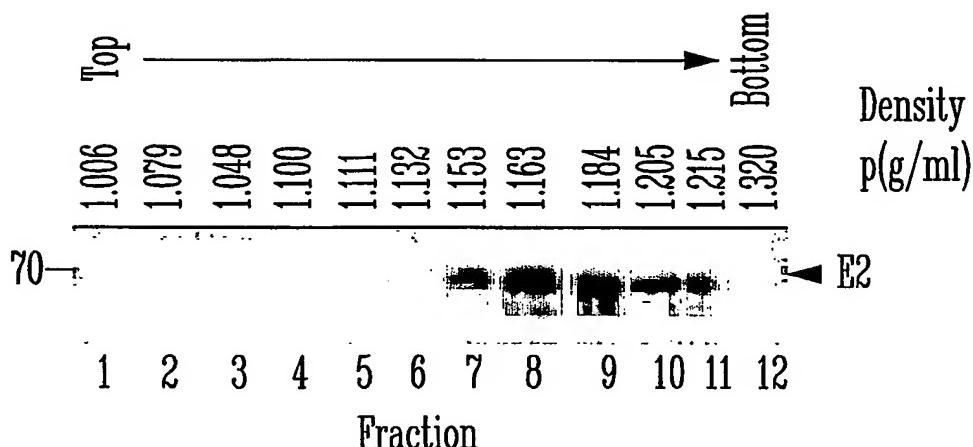


FIG - 25

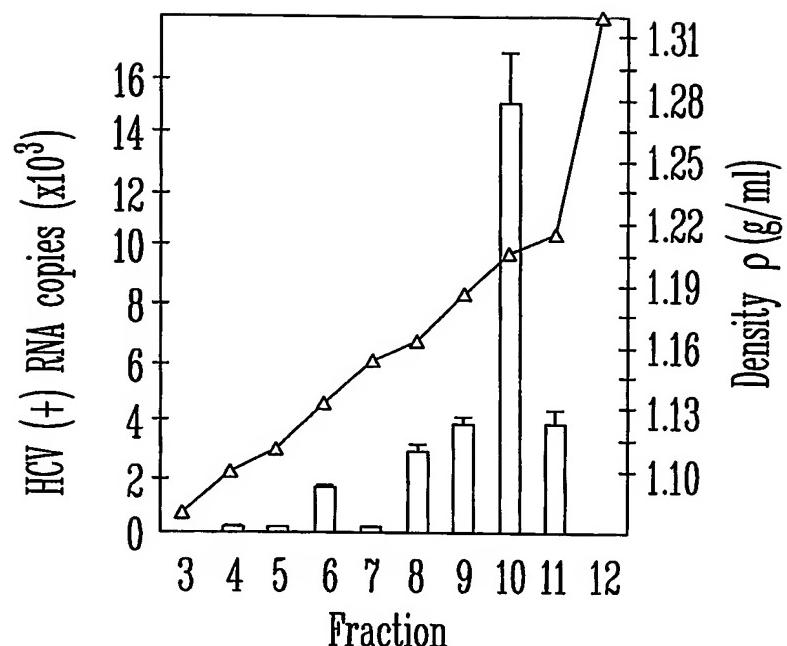
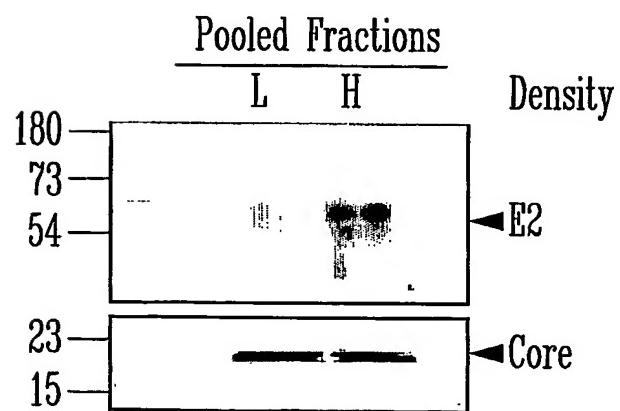
30 / 72

~~TEST - 26A~~~~TEST - 26B~~~~TEST - 26C~~

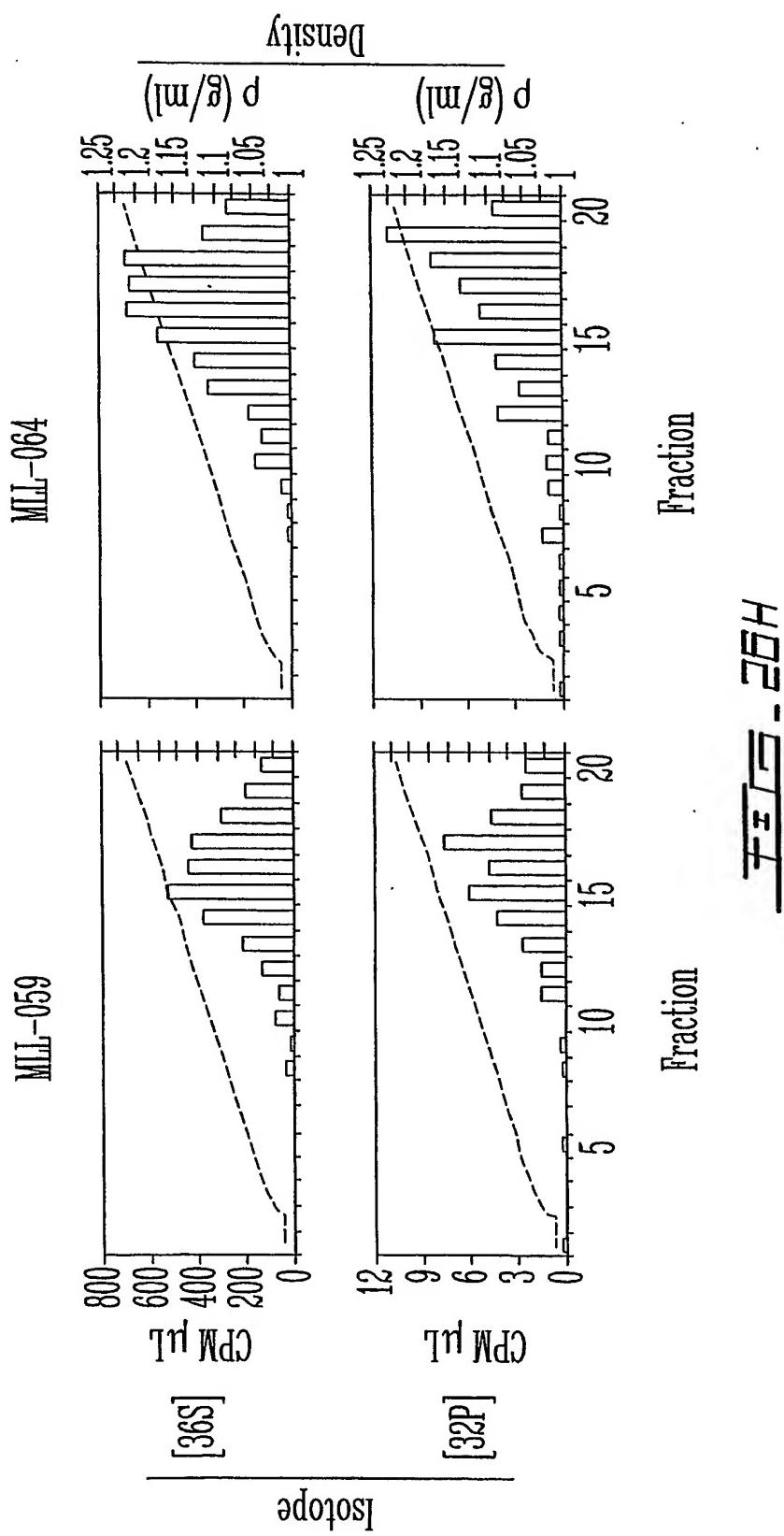
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F1G - 260F1G - 266

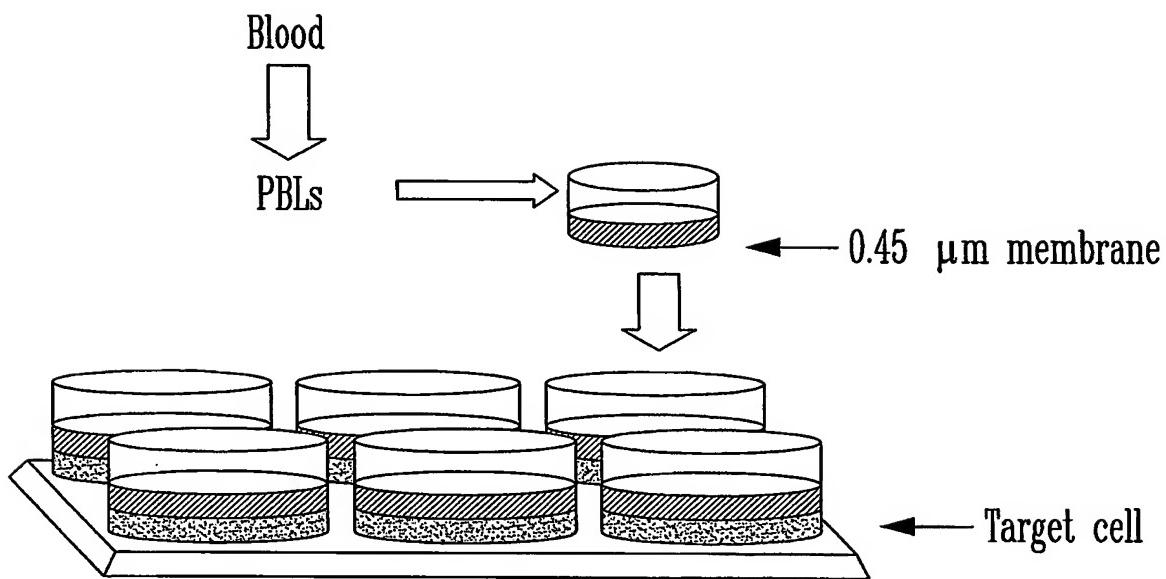
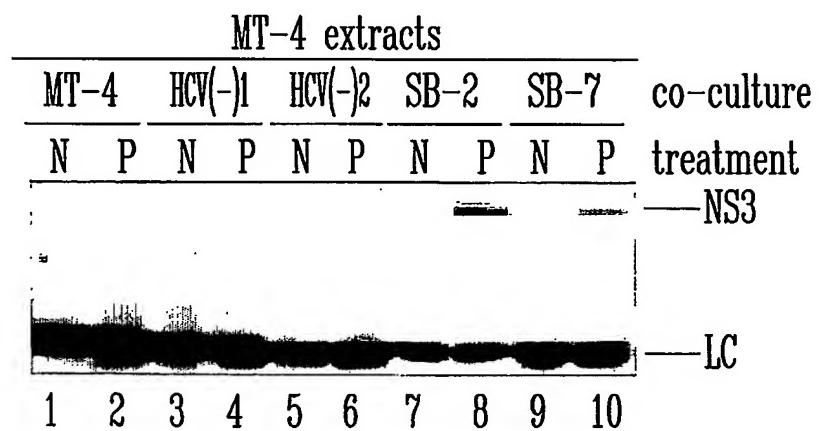
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FIG - 26FFIG - 26G

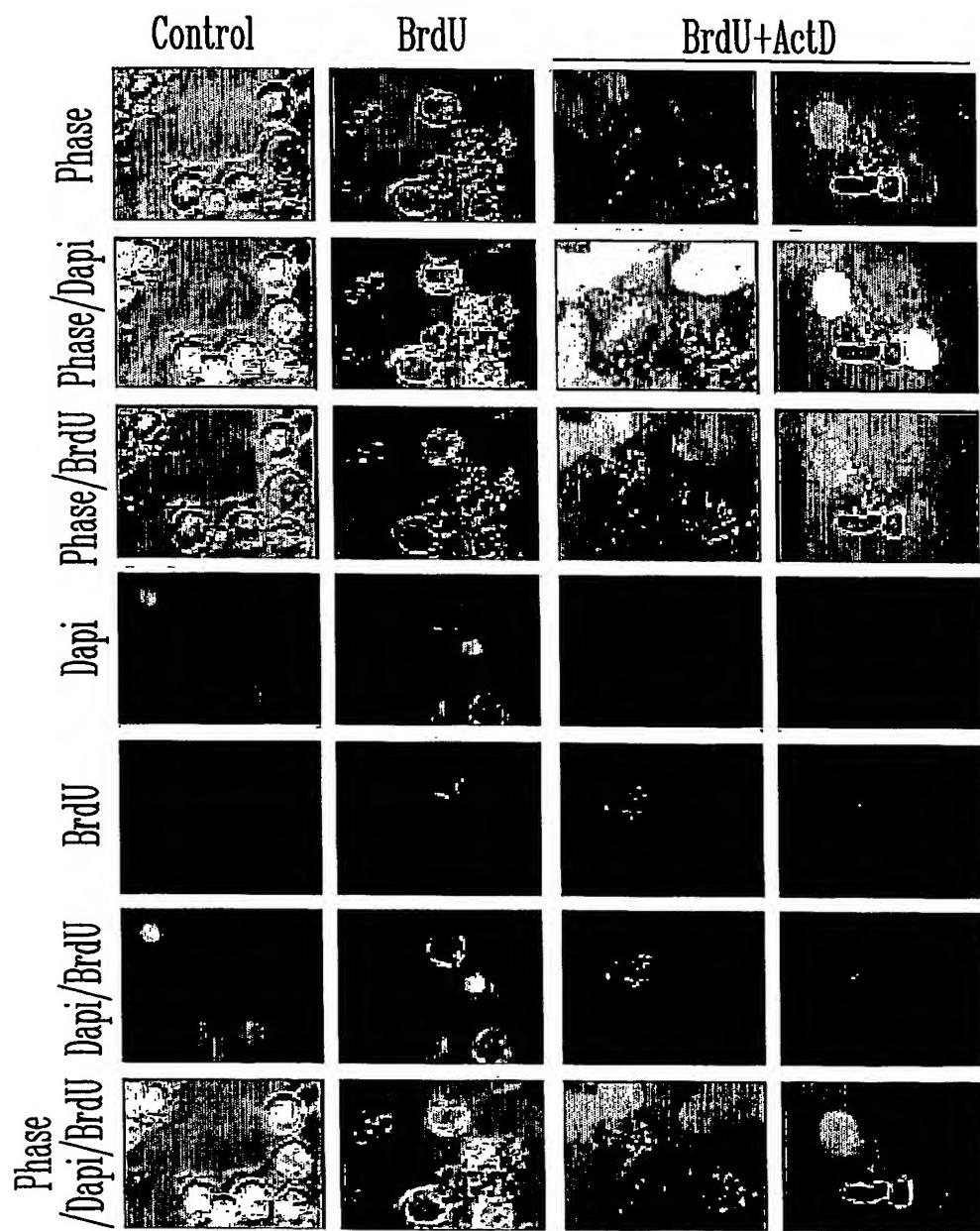
33 / 72



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FIG - 27AFIG - 27B

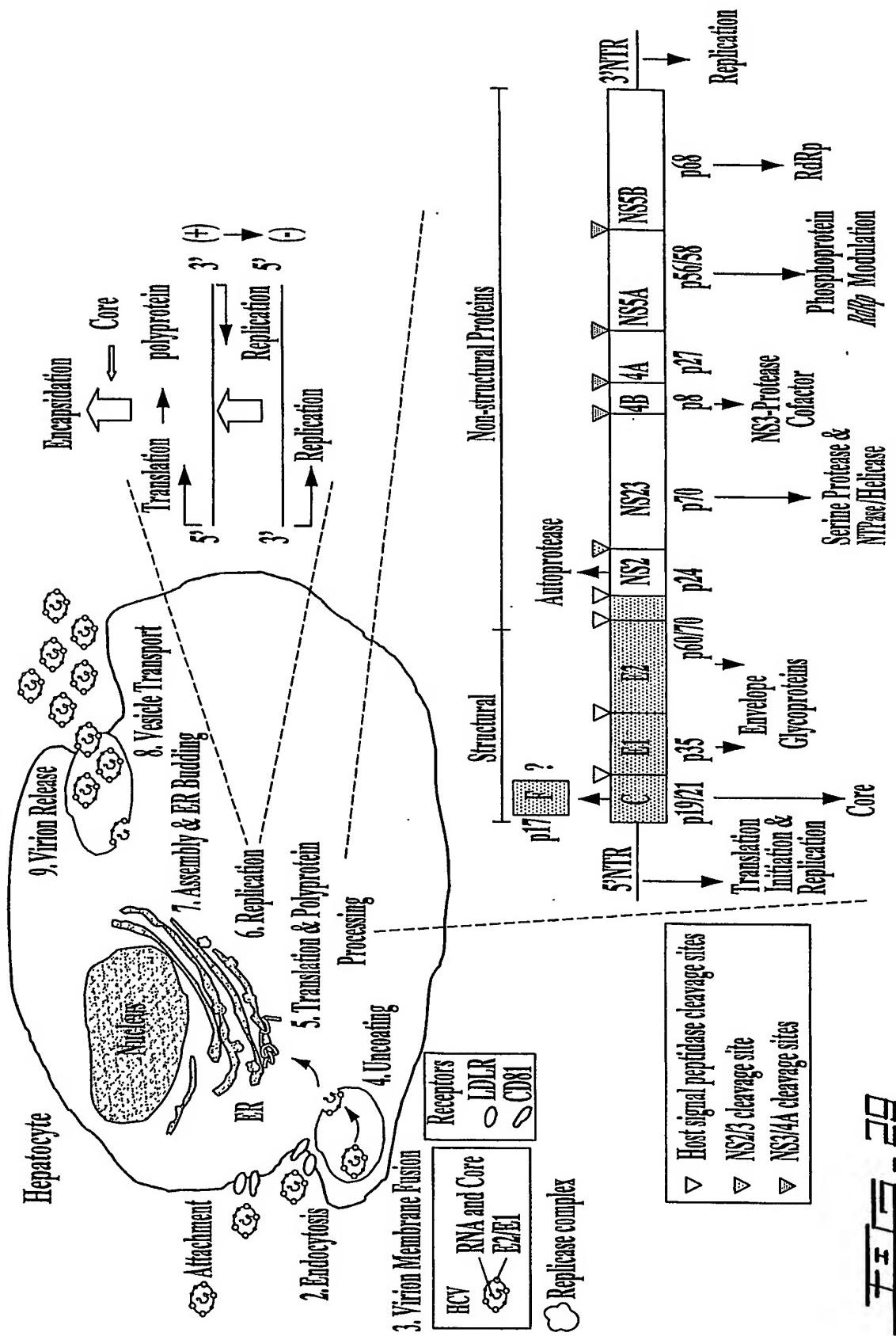
35 / 72

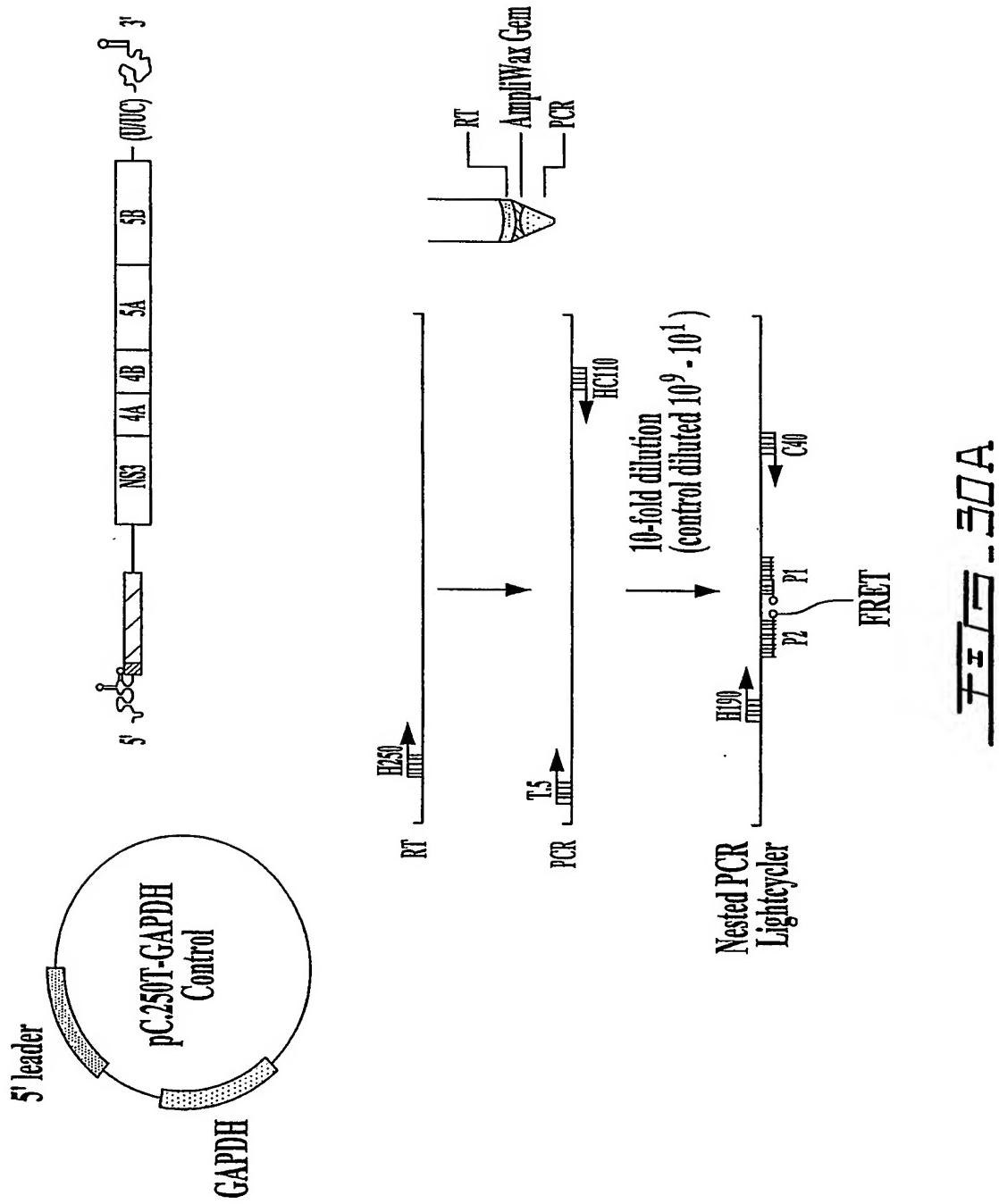


~~FIGURE - 28~~

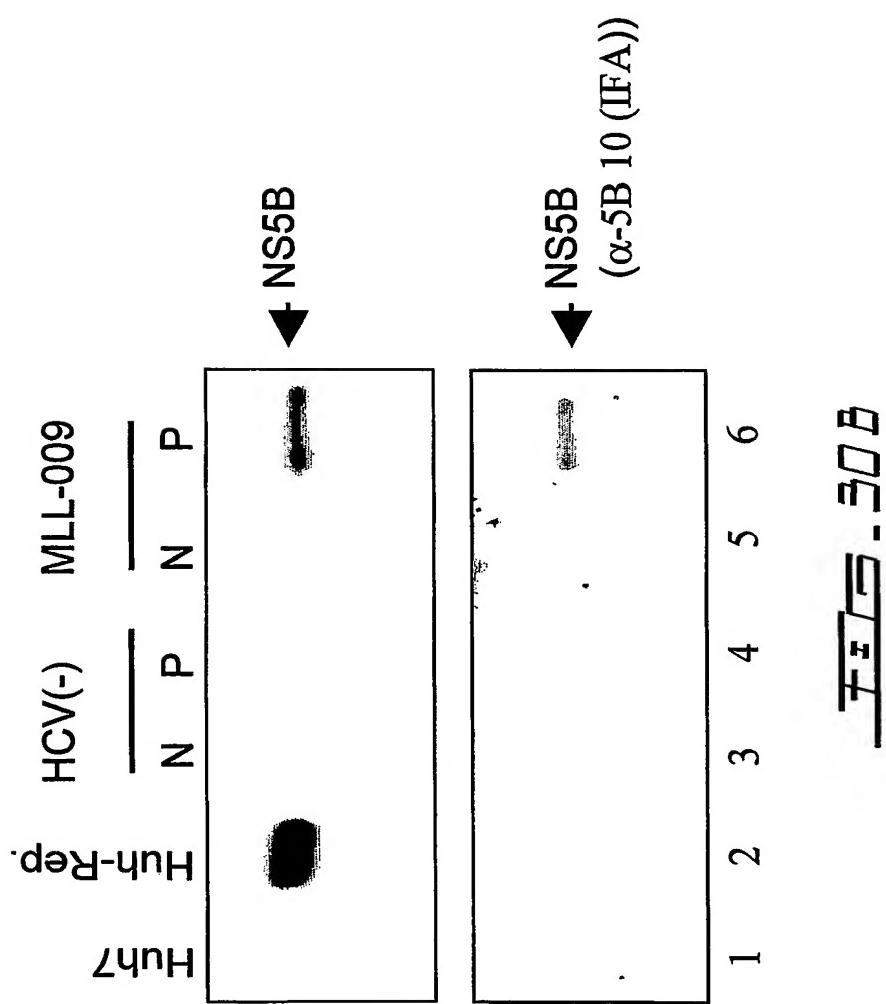
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## HCV Replication Cycle

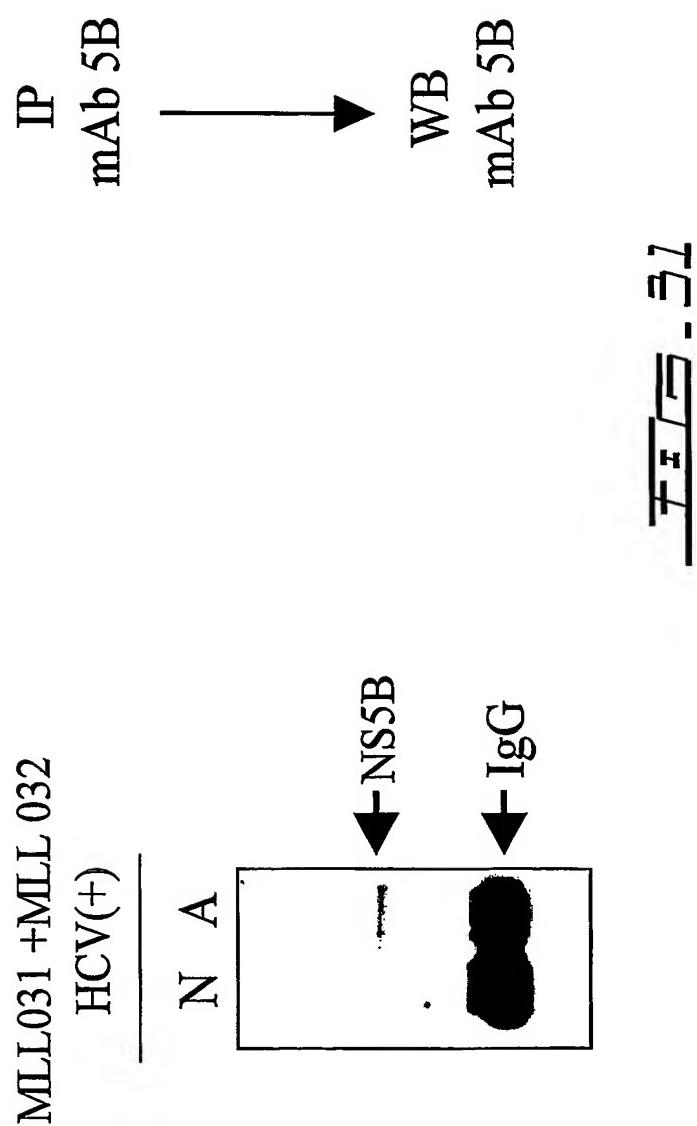




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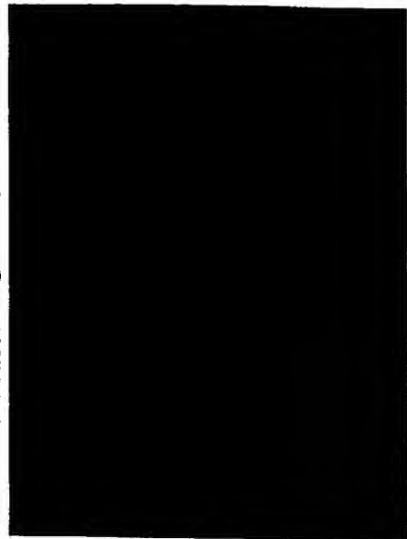
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Anti-Core



Dapi



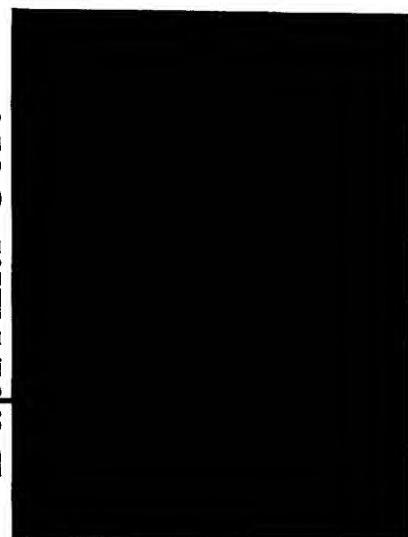
Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core



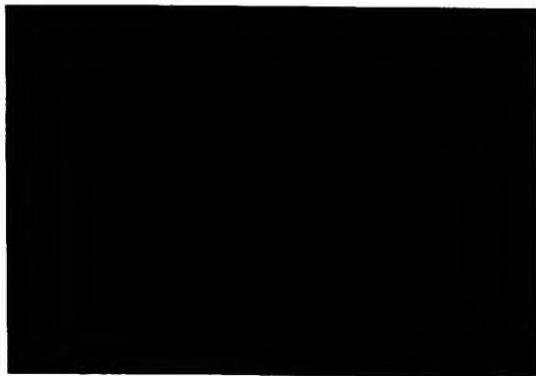
7375 - 32

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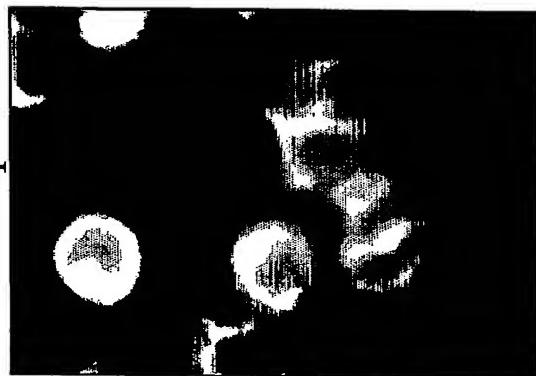
41/72

~~FIGURE 33~~

Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core

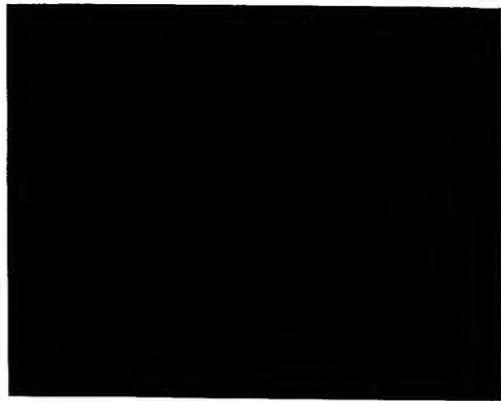


Dapi/Anti-Core

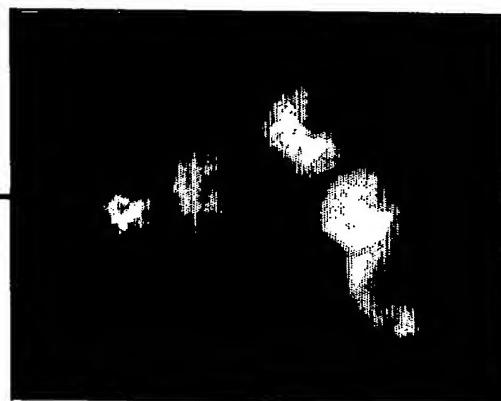


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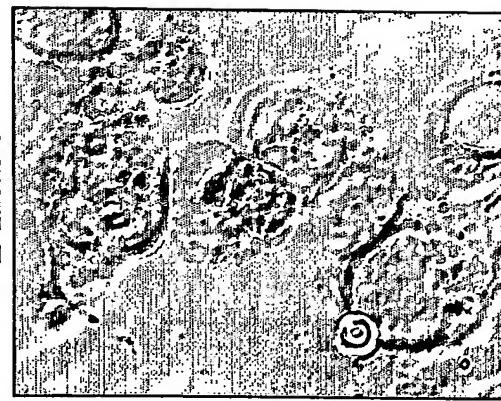
Anti-Core



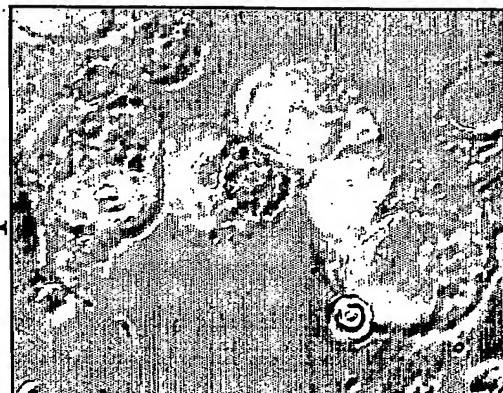
Dapi



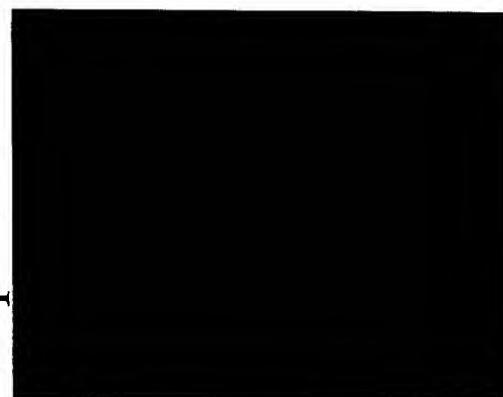
Phase



Phase/Dapi/Anti-Core



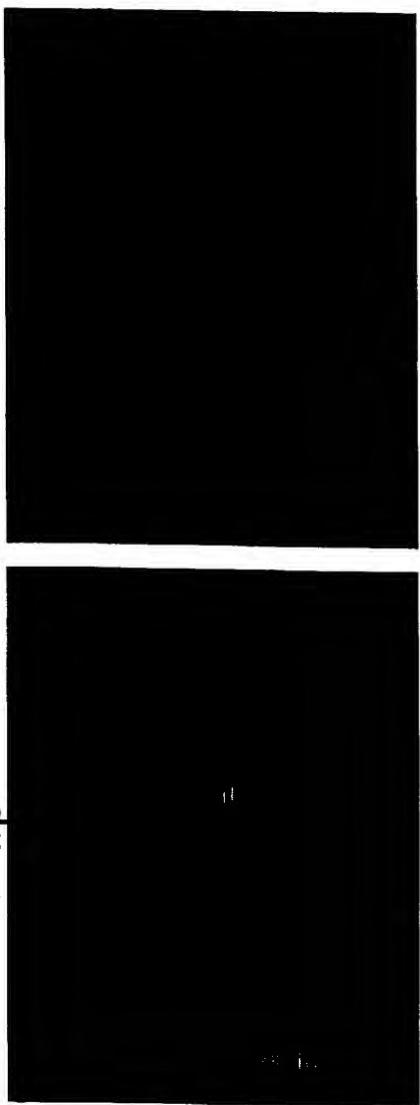
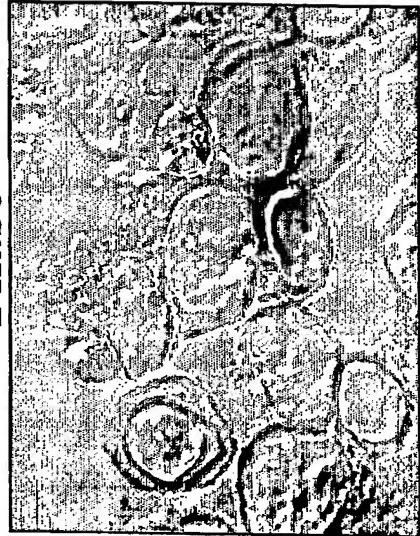
Dapi/Anti-Core



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Phase      Dapi      Anti-Core



Dapi/Anti-Core      Phase/Dapi/Anti-Core

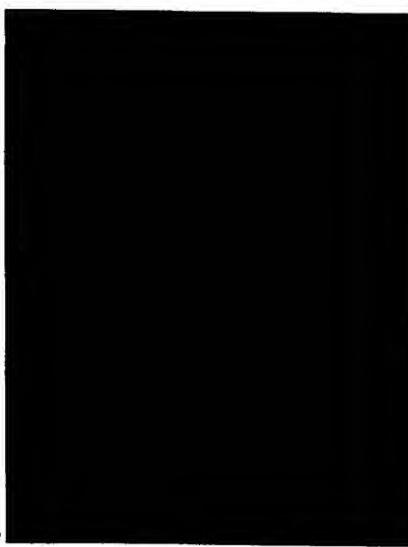
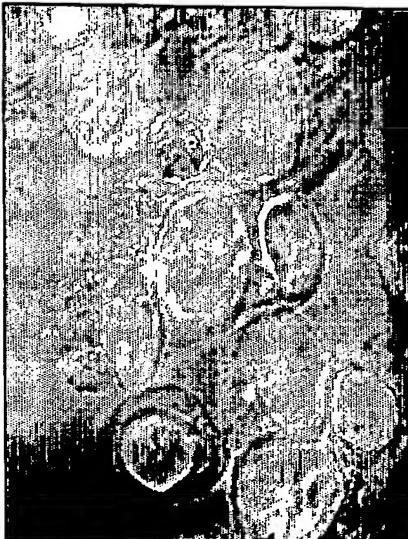


FIG. 35

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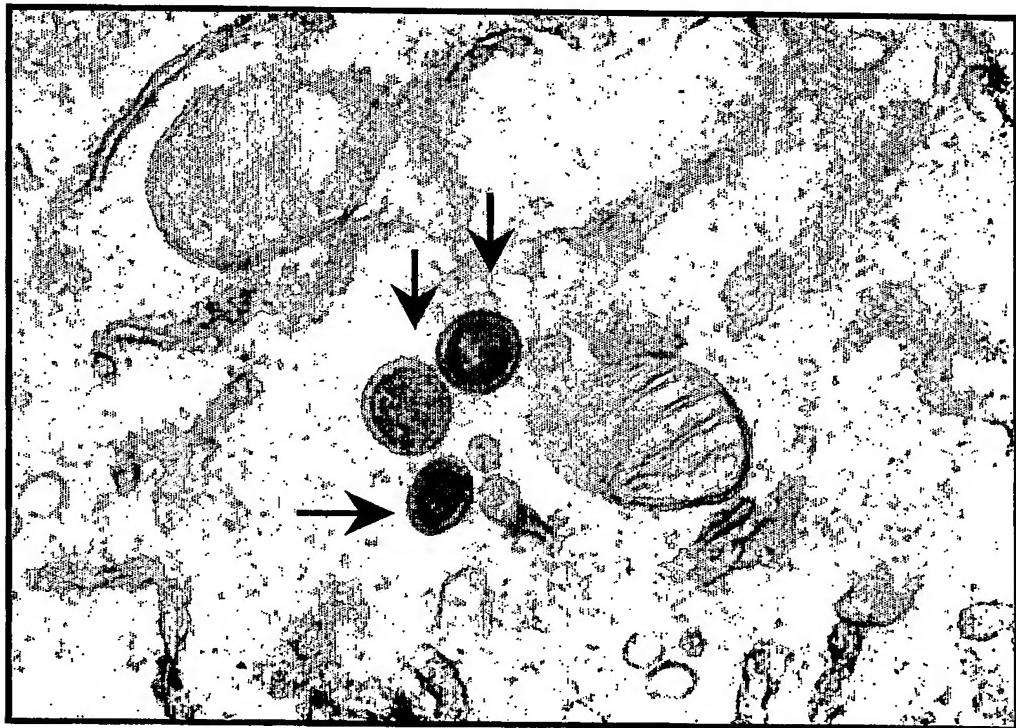
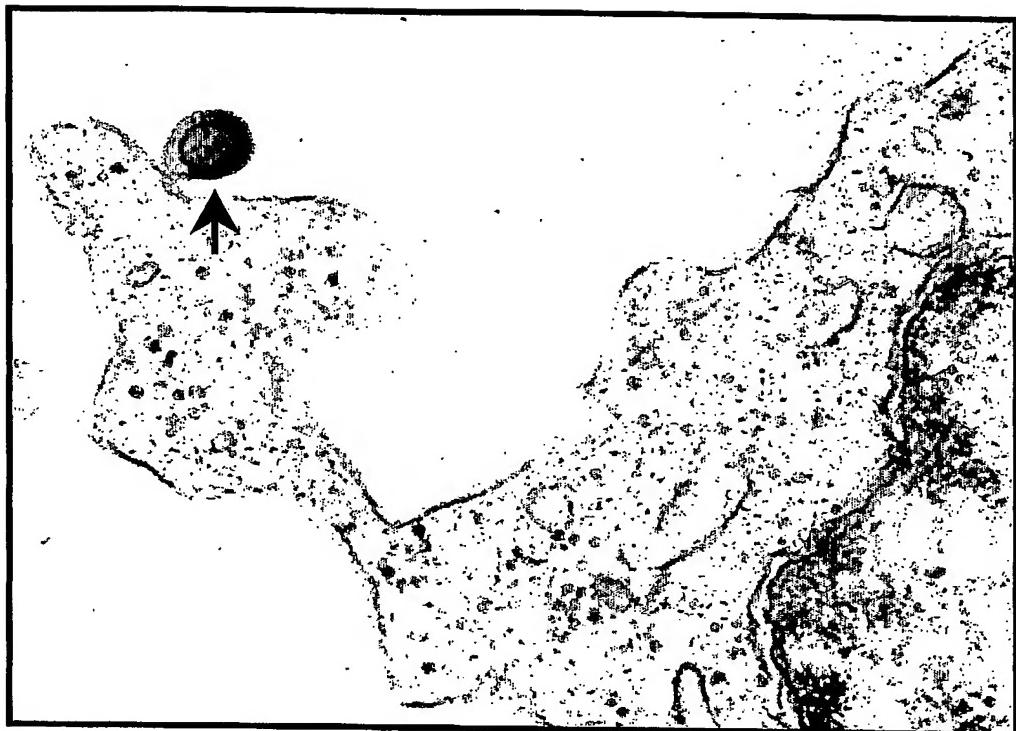
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FIG - 36 C  
FIG - 36 B  
FIG - 36 A

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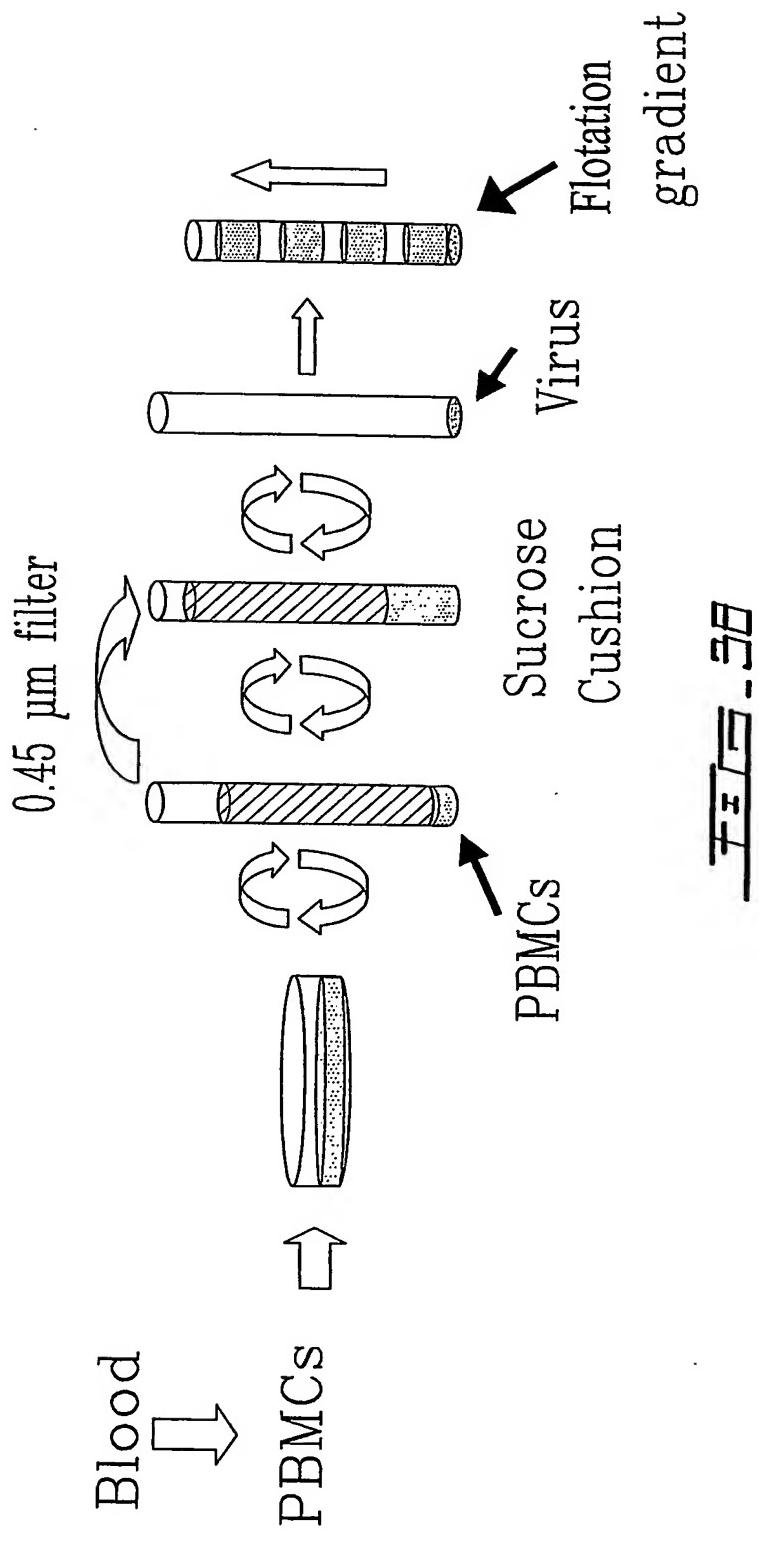
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~~FIG - 37~~

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## Virus partial purification.



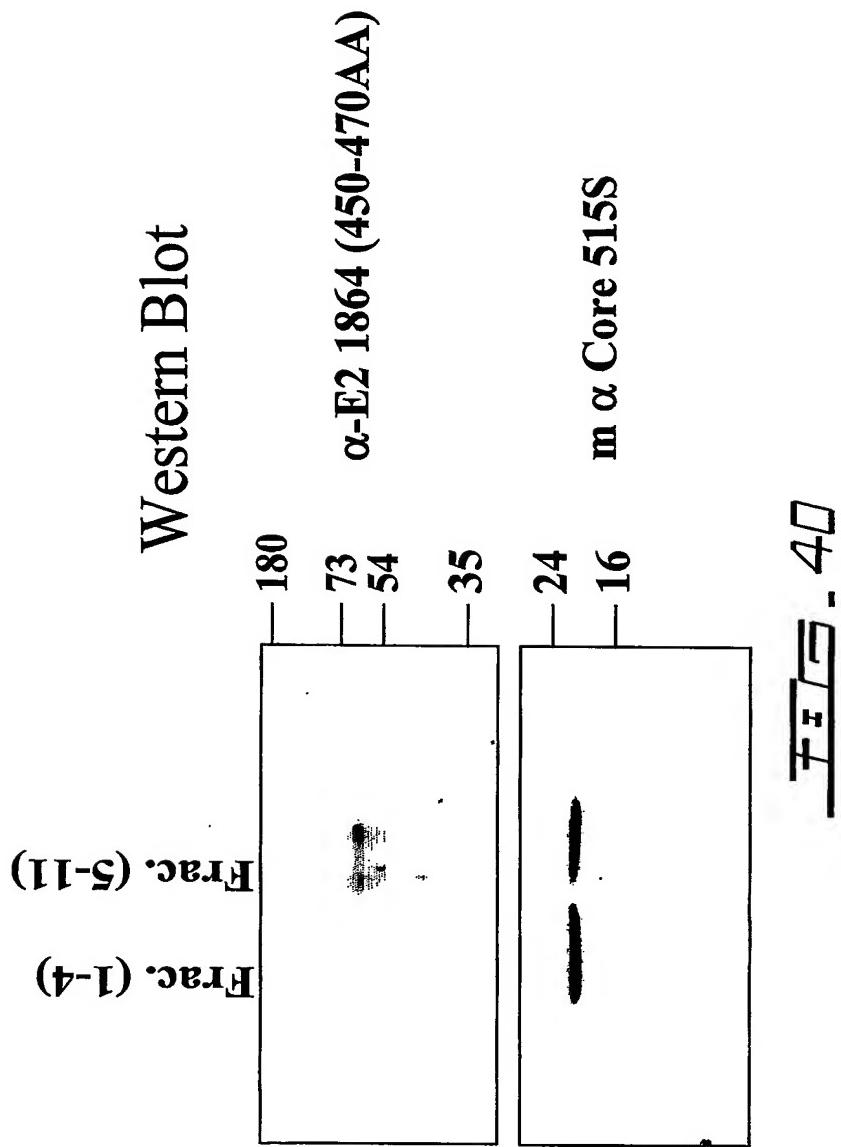
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Density Range (g/ml)	Source	Reference
1.15-1.20	HCV-LP in VSV vector	J.Virol (2002) 76, 12325.
1.14-1.18	HCV-LP in insect cells	J. Virol (1998) 72, 3827.
1.12-1.17	Plasma chimps	J. Gen. Virol (1994) 75, 1755
1.09-1.21	Plasma chimps	J. Med. Virol (1991), 34, 206.
1.13-1.17	Plasma chimps	J. Virol (1993) 67, 1953
1.063-1.21	Serum infected donors	J. Med. Virol (2002) 68, 335
1.11-1.215	HCV(+) PBMCS	-----

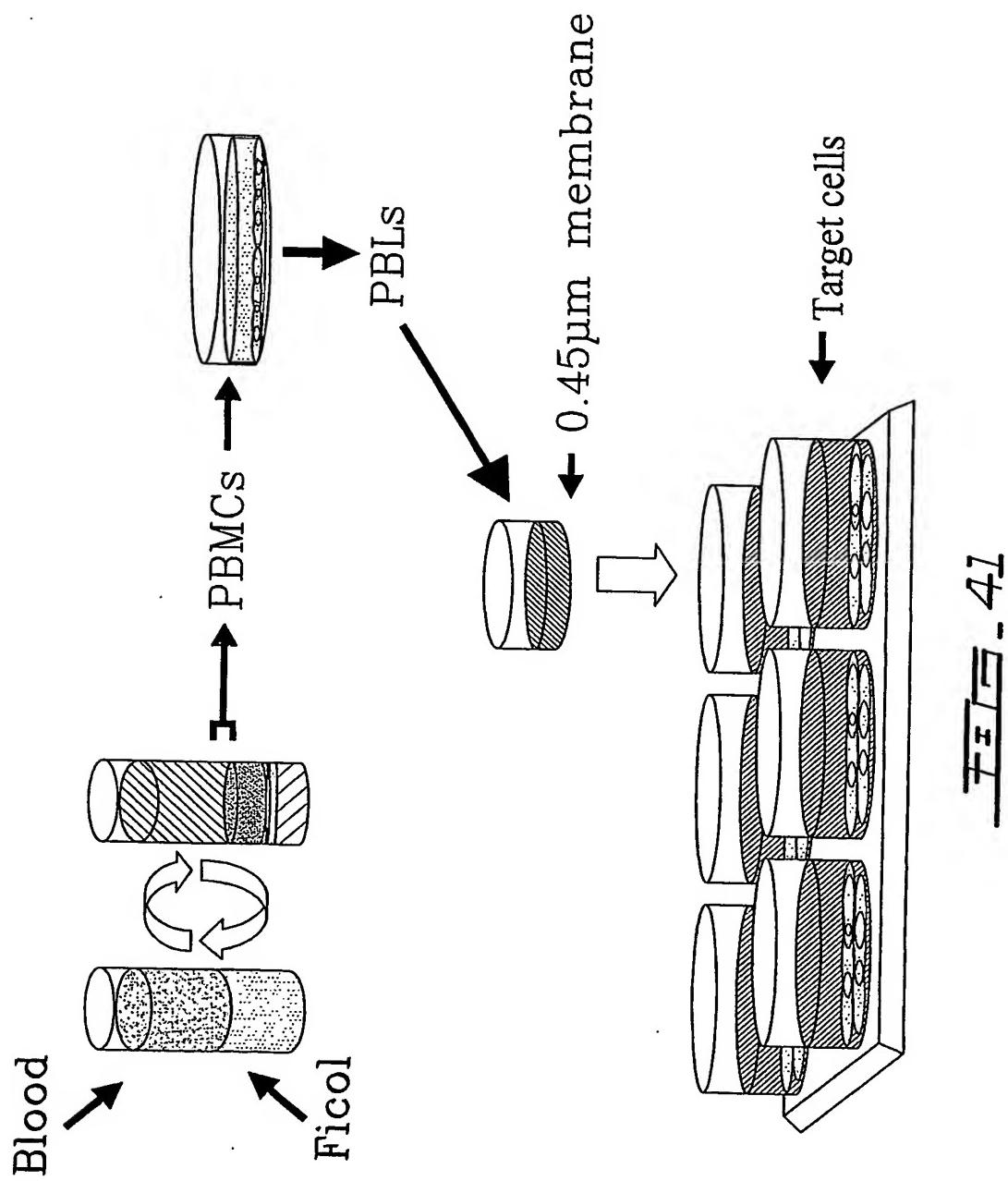
~~7-17-05~~ - 39

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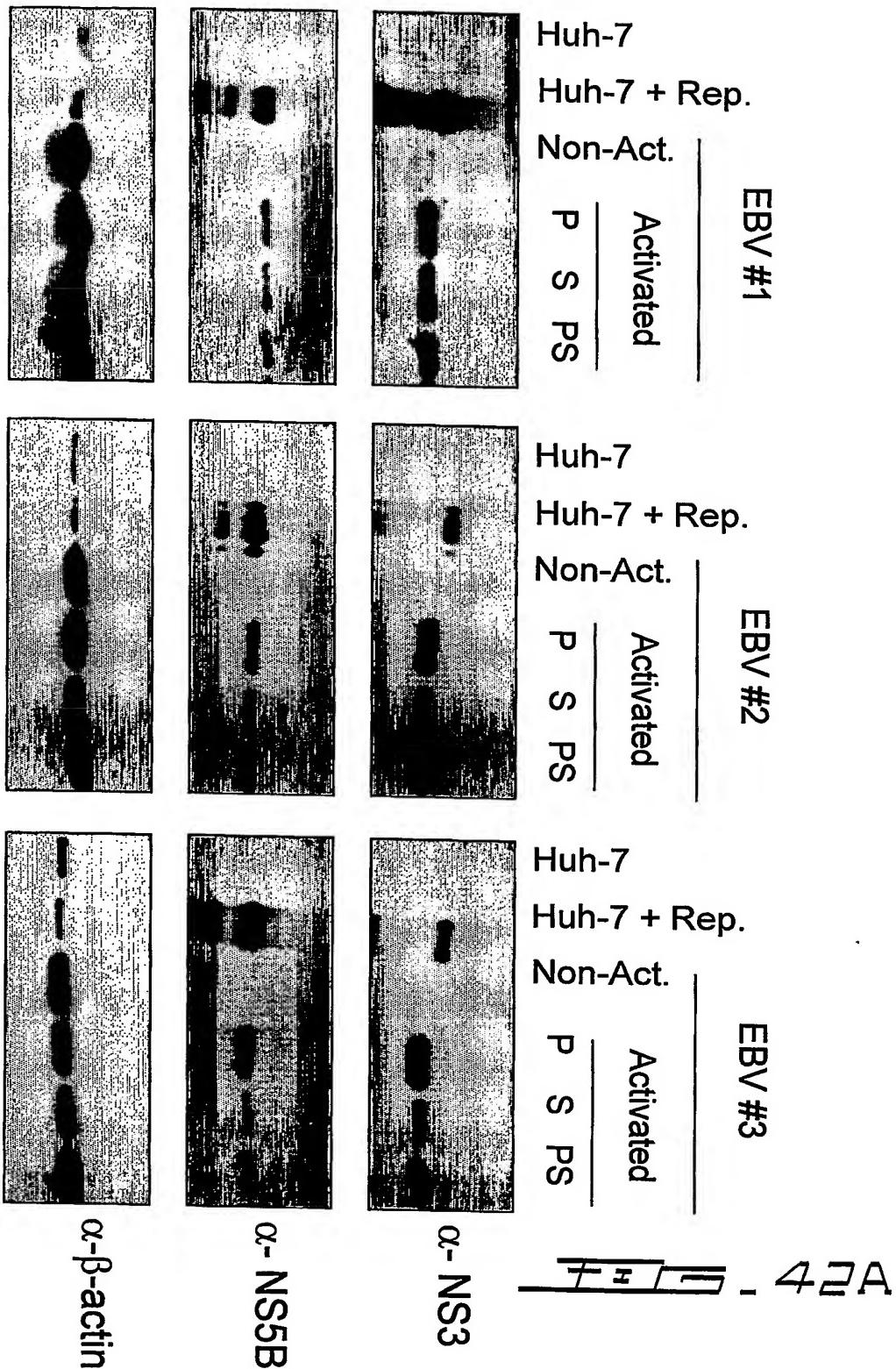
## Western Blot



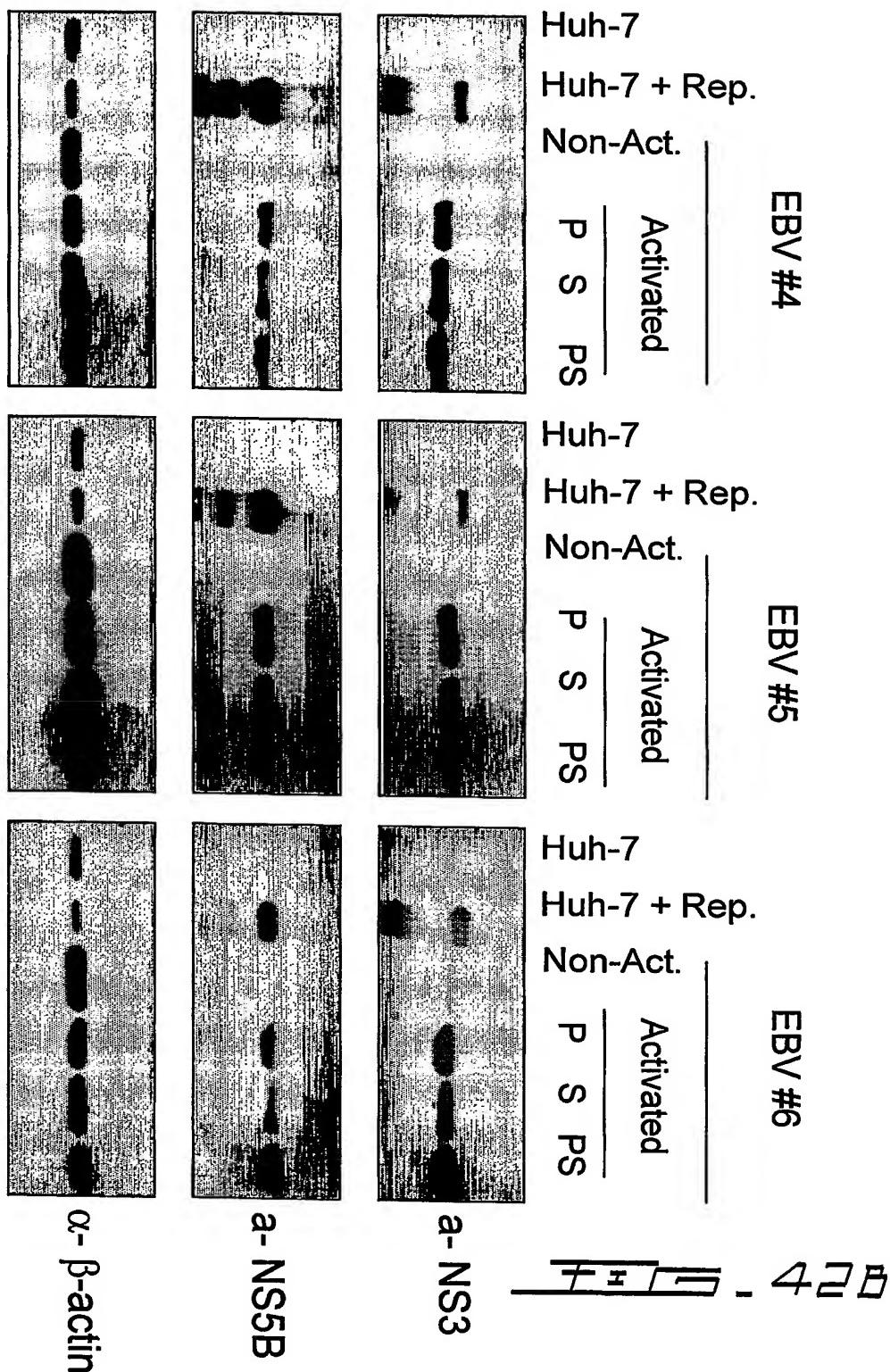
49 / 72

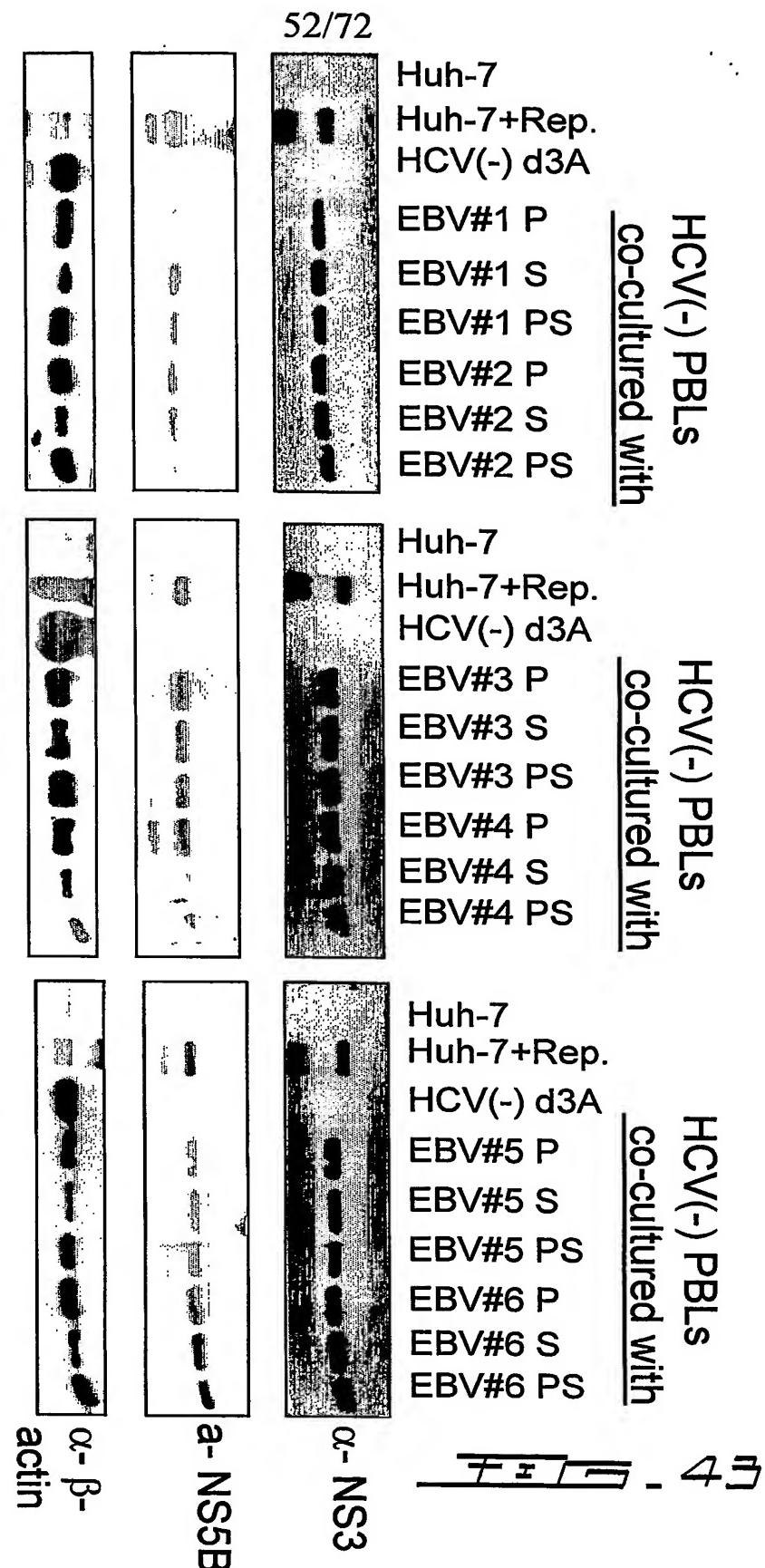


50/72

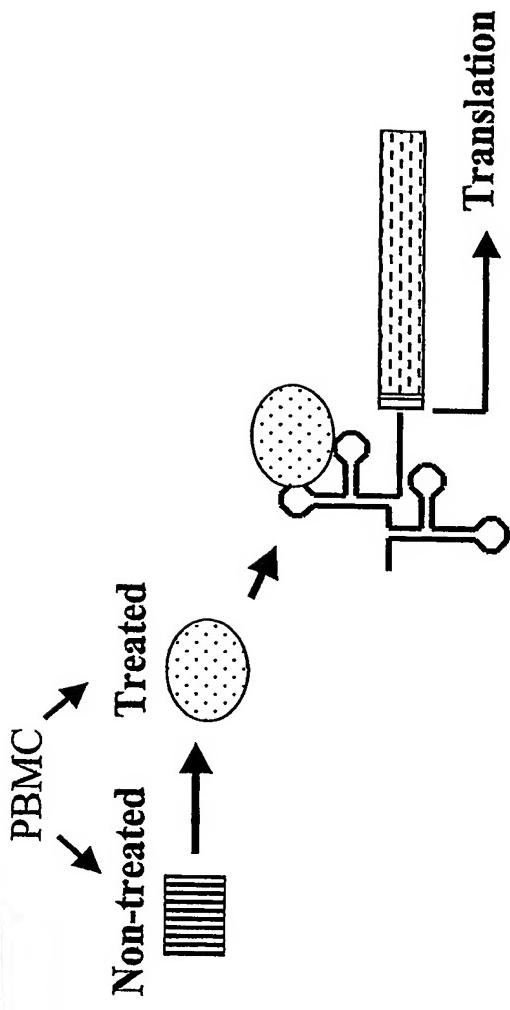


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I- Translation Activator.



II- Translation inhibitor.

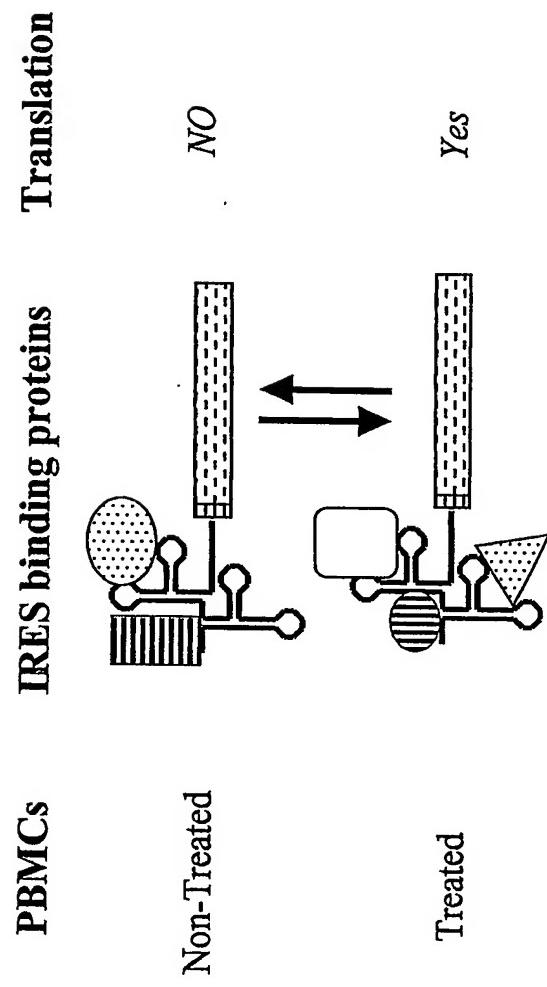
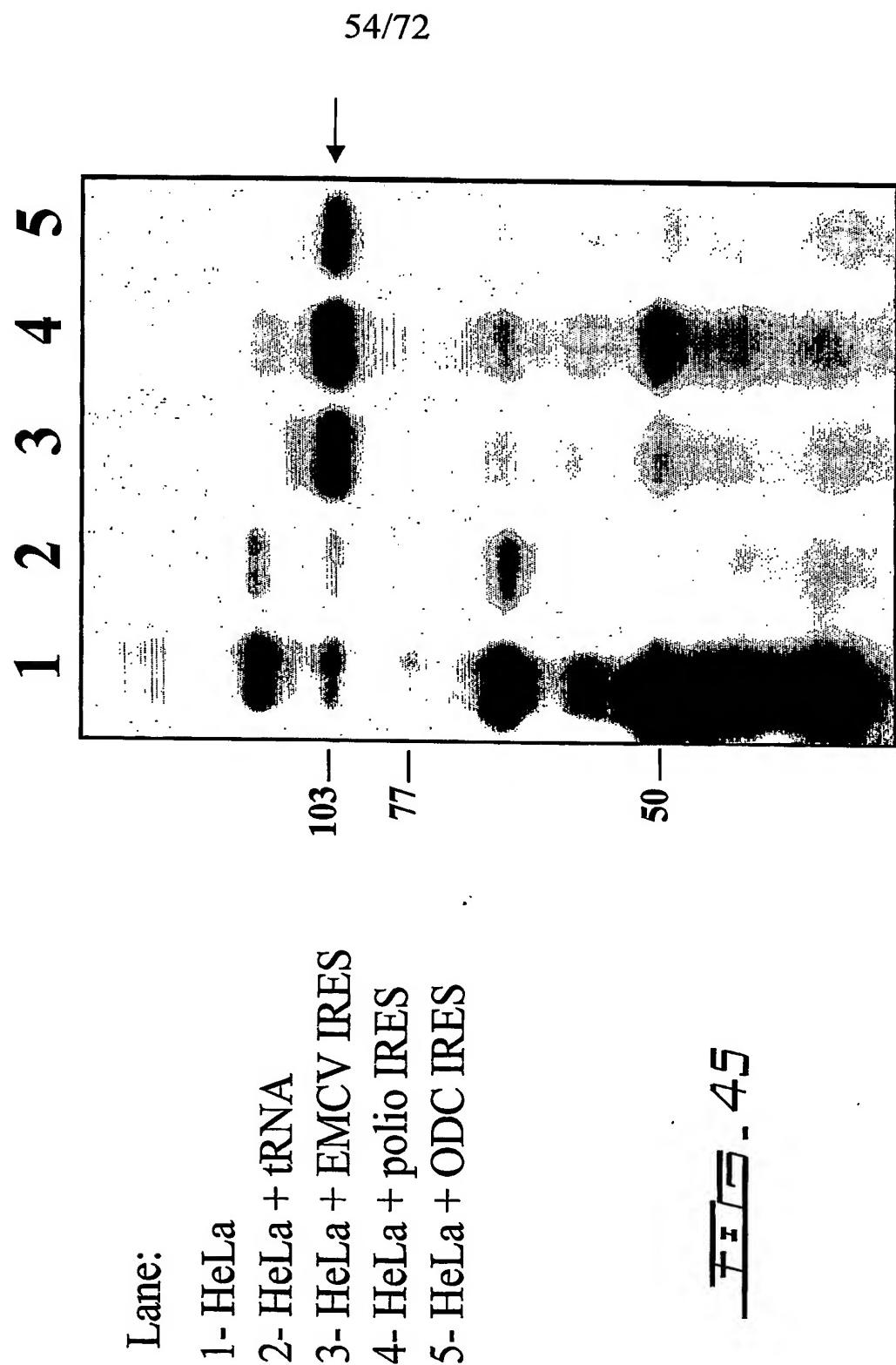
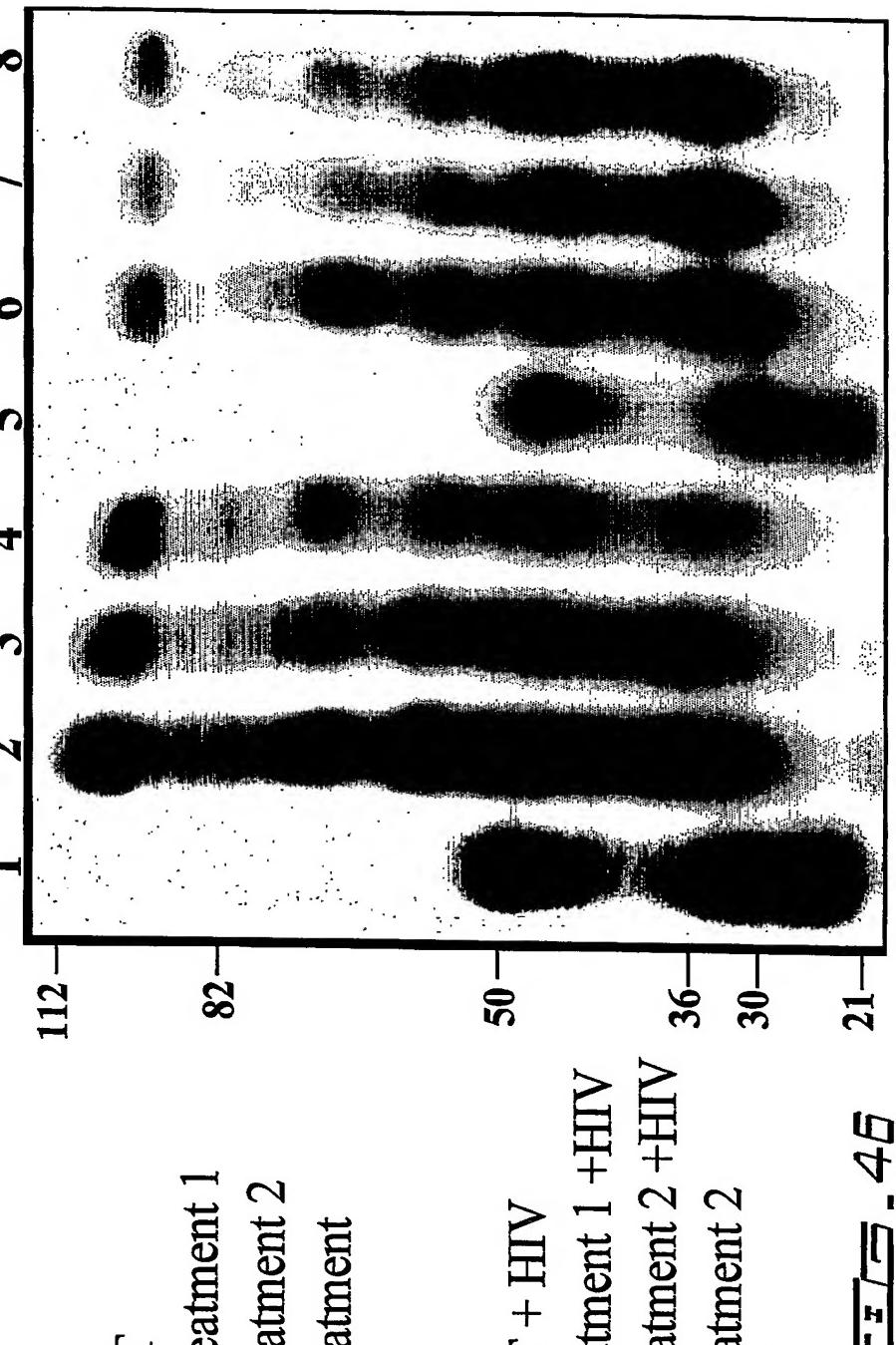


FIGURE - 44



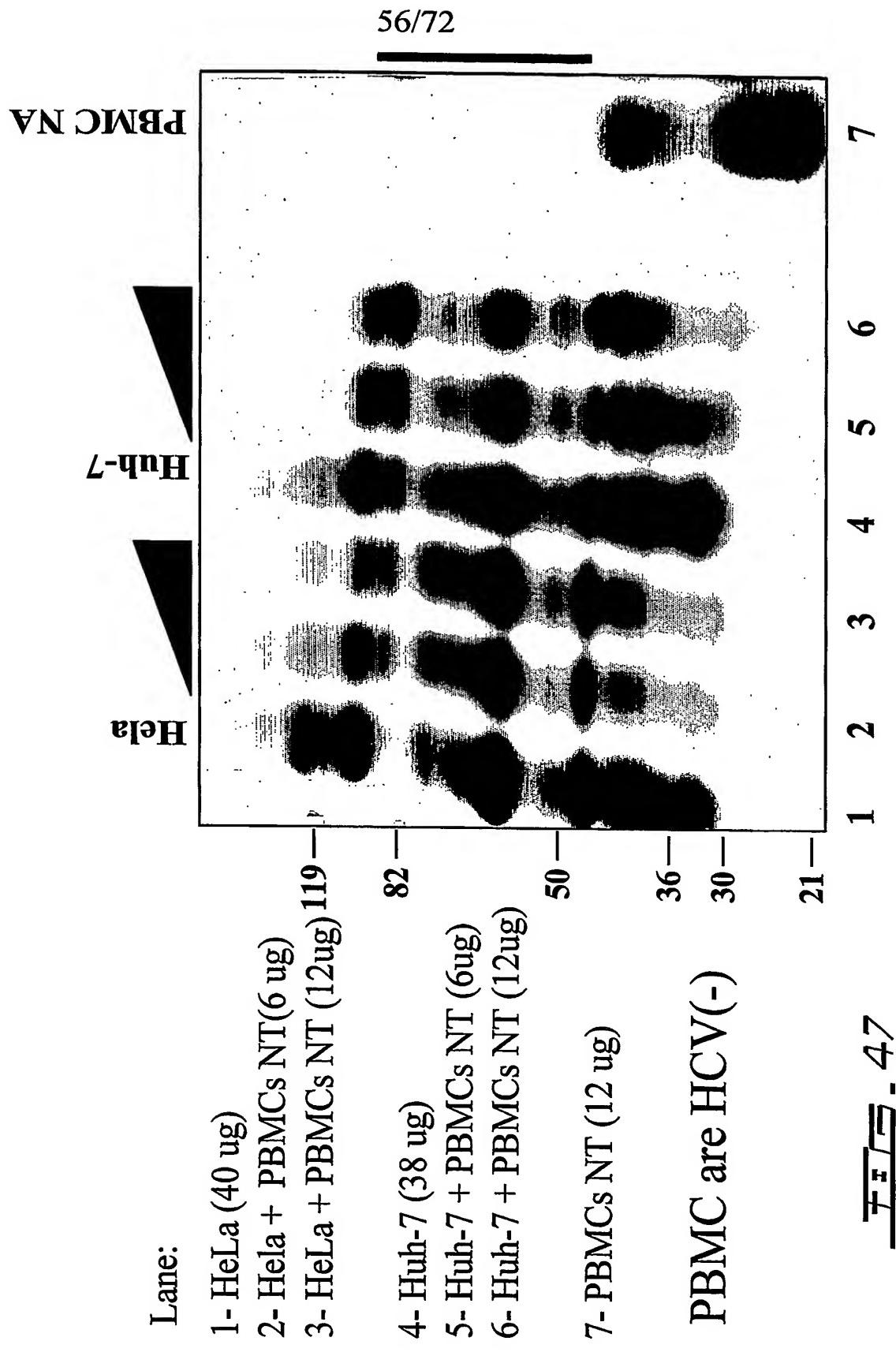
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7.5/5 - 4E

Lane:

- 1- PBMCs NT
- 2- PBMCs treatment 1
- 3- PBMCs treatment 2
- 4- PBMCs treatment  
2+DEVA

- 5- PBMCs NT + HIV
- 6-PBMCs treatment 1 +HIV
- 7- PBMCs treatment 2 +HIV
- 8- PBMCs treatment 2  
+DEVA+HIV



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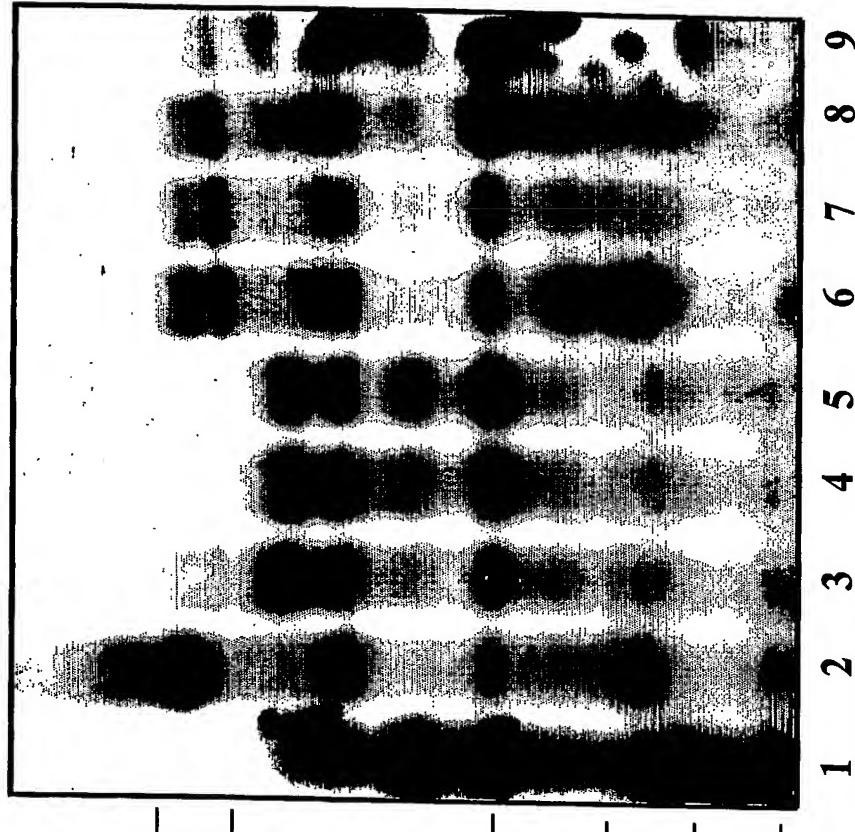
Huh-7

HeLa

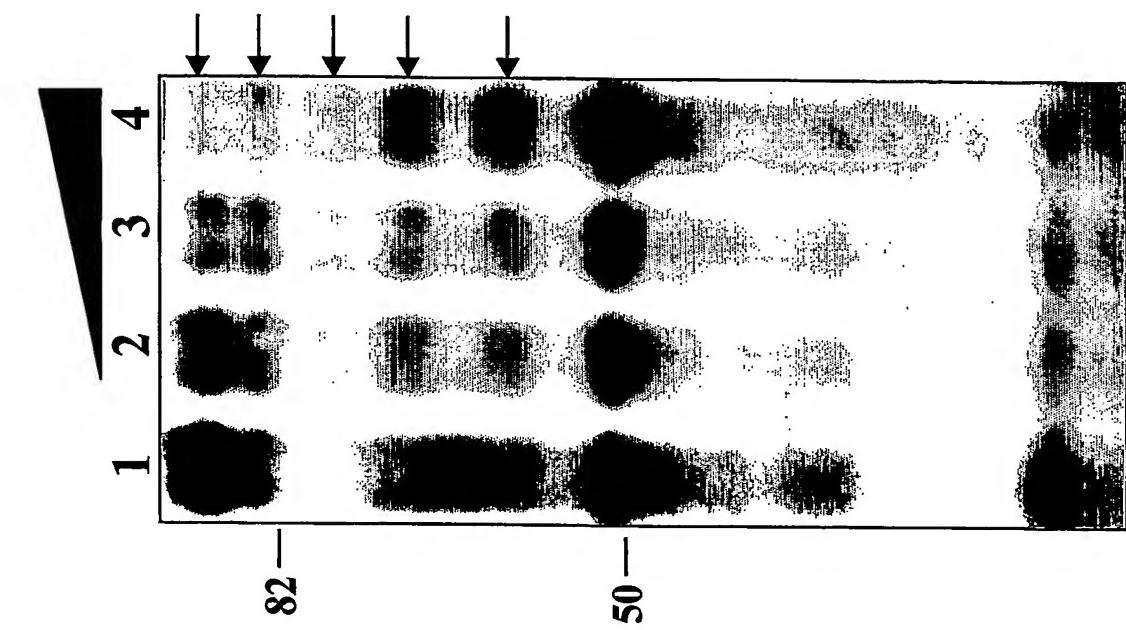
Lane:

1- HCV (-) PBMCS NA (20 ug)

- 2- HeLa (20 ug)
- 3- HeLa + HCV (-) PBMCS NT (5 ug)
- 4- HeLa + HCV (-) PBMCS NT (10 ug)
- 5- HeLa + HCV (-) PBMCS NT (20 ug)
- 6- Huh-7 (20 ug)
- 7- Huh-7 + HCV (-) PBMCS NT (5 ug)
- 8- Huh-7 + HCV (-) PBMCS NT (10 ug)
- 9- Huh-7 + HCV (-) PBMCS NT (20 ug)

~~FIGURE - 4E~~

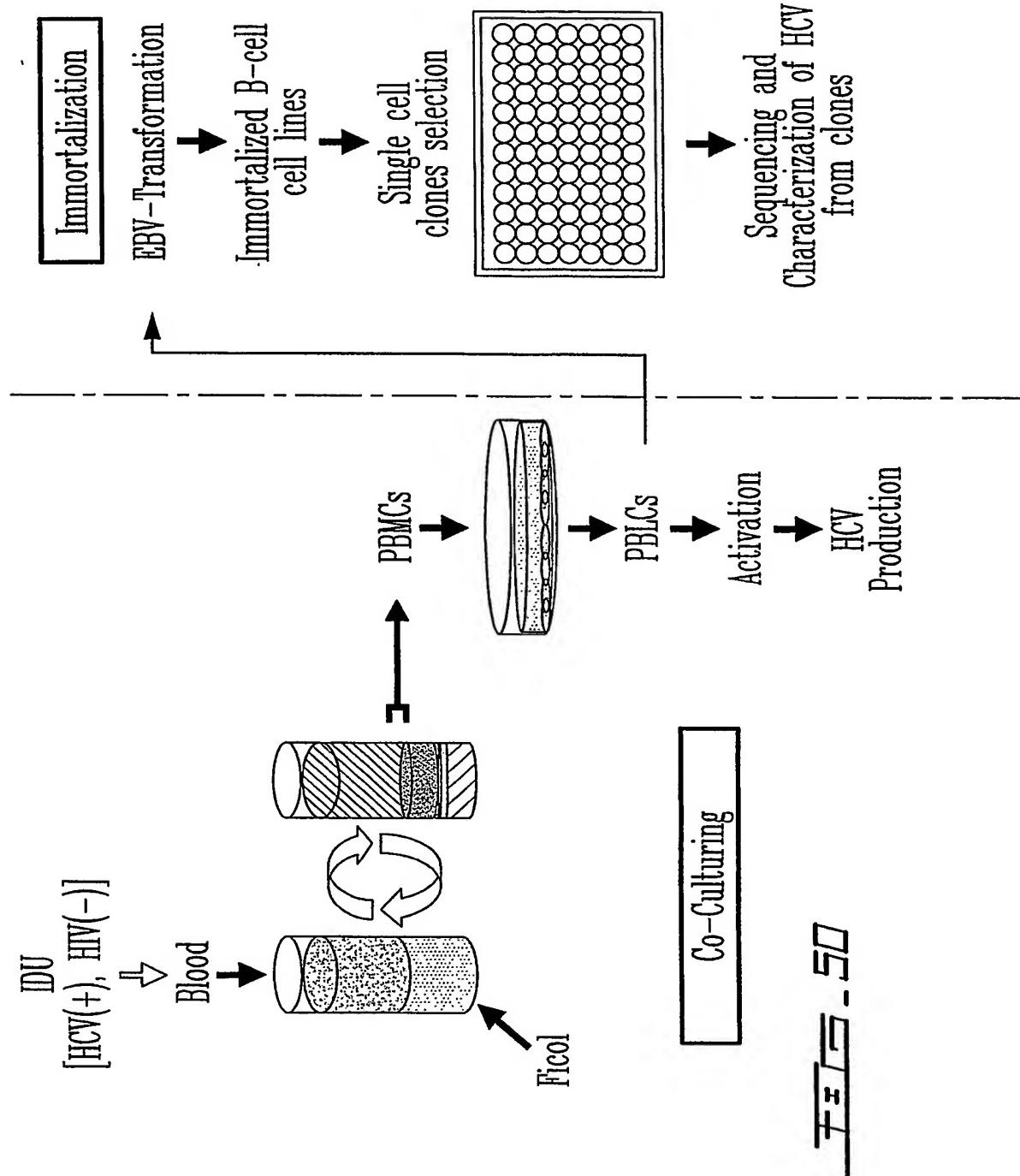
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Lane:

- 1- Huh-7 (20ug)
- 2- Huh-7 + HCV (-) PBMCS NT (5ug)
- 3- Huh-7 + HCV (-) PBMCS NT (10ug)
- 4- Huh-7 + HCV (-) PBMCS NT (20ug)

~~7575 - 49~~



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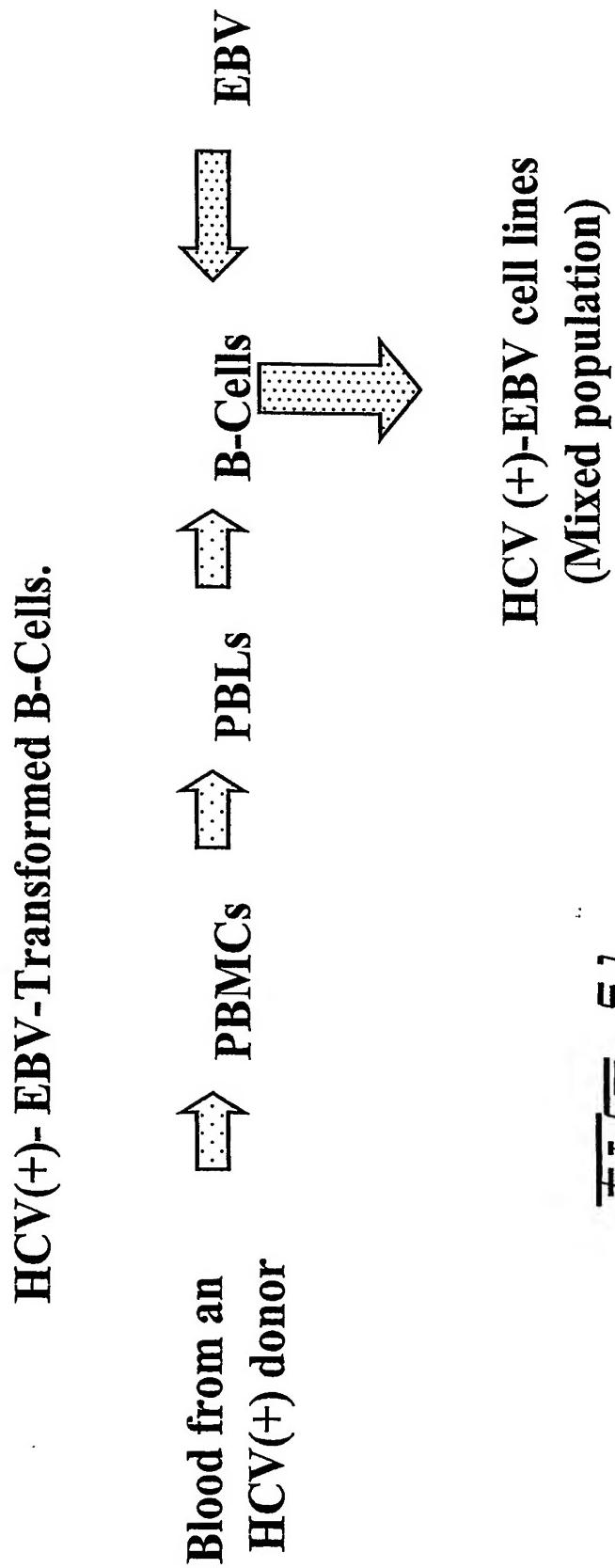


FIGURE - 51

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HCV RNA is detected in mixed population of EBV-transformed B-cells

**HCV (+) Strand RNA**

Cell line	Non-Stimulated cells RNA Copies /10 <sup>6</sup> cells	Stimulated cells RNA Copies /10 <sup>6</sup> cells
EBV-1	4.66x10 <sup>5</sup>	2.33x10 <sup>6</sup>
EBV-2	2.77x10 <sup>5</sup>	7.91x10 <sup>4</sup>
EBV-3	3.96x10 <sup>6</sup>	4.02x10 <sup>5</sup>
EBV-4	2.03x10 <sup>6</sup>	1.57x10 <sup>6</sup>
EBV-6	1.41x10 <sup>6</sup>	4.32x10 <sup>5</sup>
EBV-HCV (-)	0	0

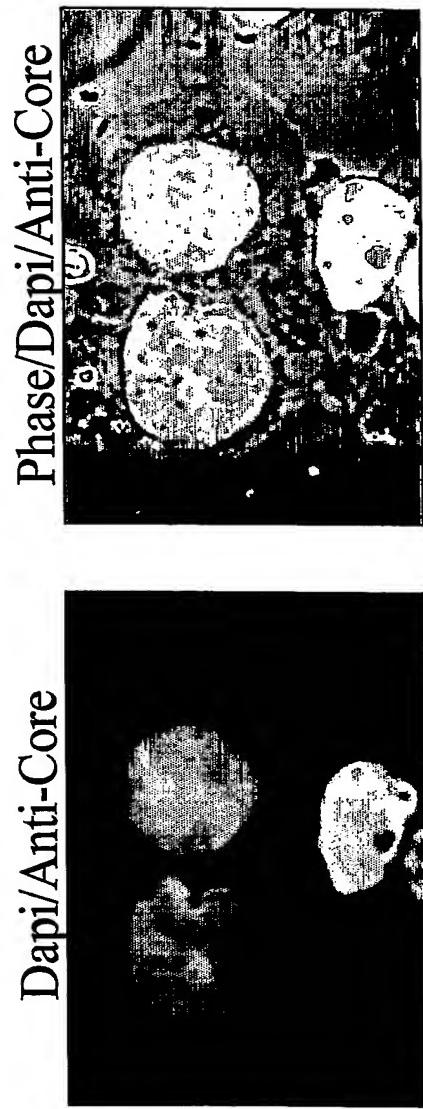
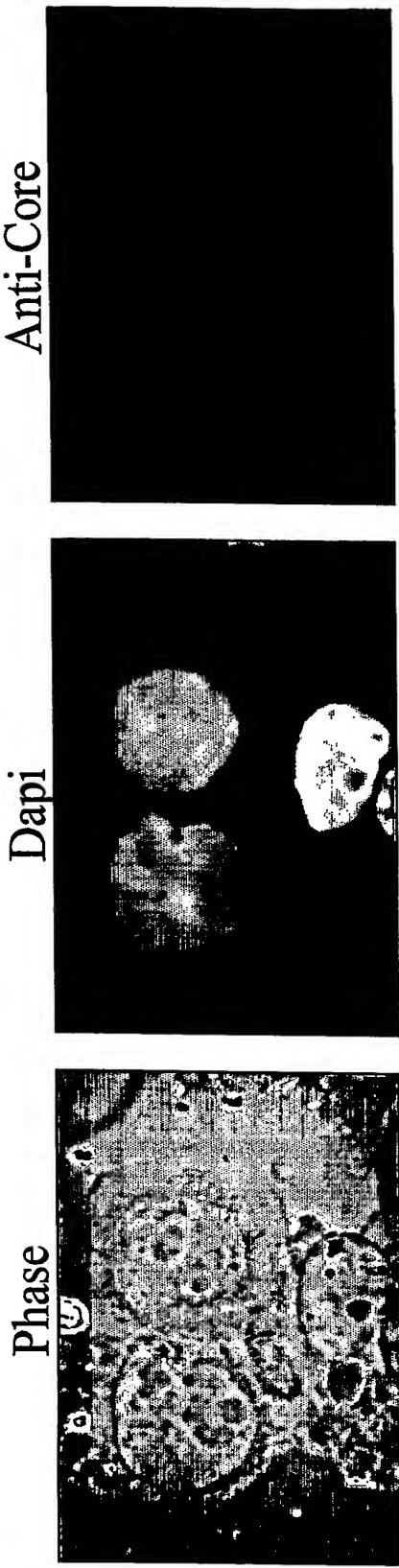
**GAPDH mRNA**

Cell line	Non-Stimulated cells RNA Copies /10 <sup>6</sup> cells	Stimulated cells RNA Copies /10 <sup>6</sup> cells
EBV-1	2.23x10 <sup>8</sup>	2.19x10 <sup>8</sup>
EBV-2	8.73x10 <sup>8</sup>	2.25x10 <sup>8</sup>
EBV-3	1.83x10 <sup>9</sup>	1.77x10 <sup>9</sup>
EBV-4	5.48x10 <sup>8</sup>	3.79x10 <sup>8</sup>
EBV-6	1.26x10 <sup>9</sup>	9.42x10 <sup>8</sup>
EBV-HCV (-)	9.27x10 <sup>7</sup>	3.62x10 <sup>8</sup>

7E/7E - 52

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## Control EBV-HCV (-); anti-Core



737 - 53A

63/72

Detection of Core in EBV-2  
Phase  
Dapi  
Anti-Core



Phase/Dapi/Anti-Core  
Dapi/Anti-Core

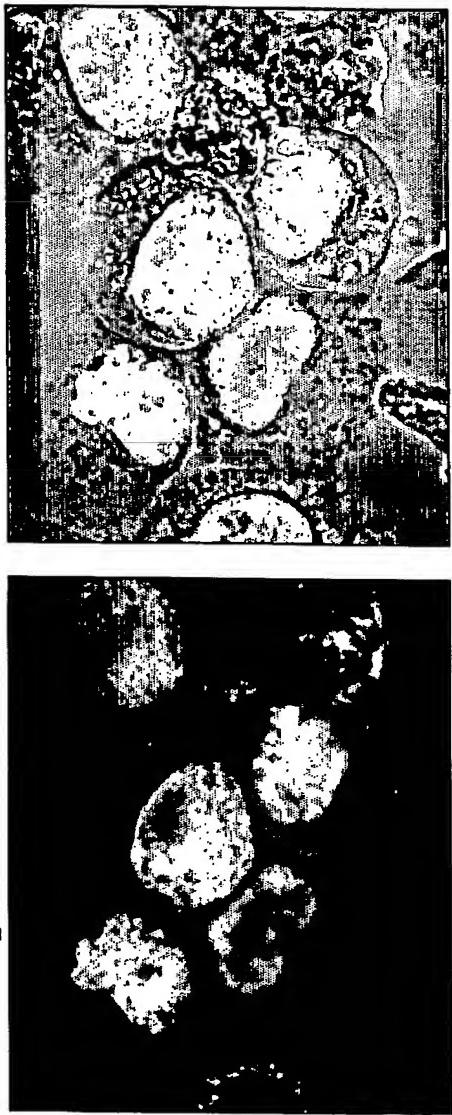
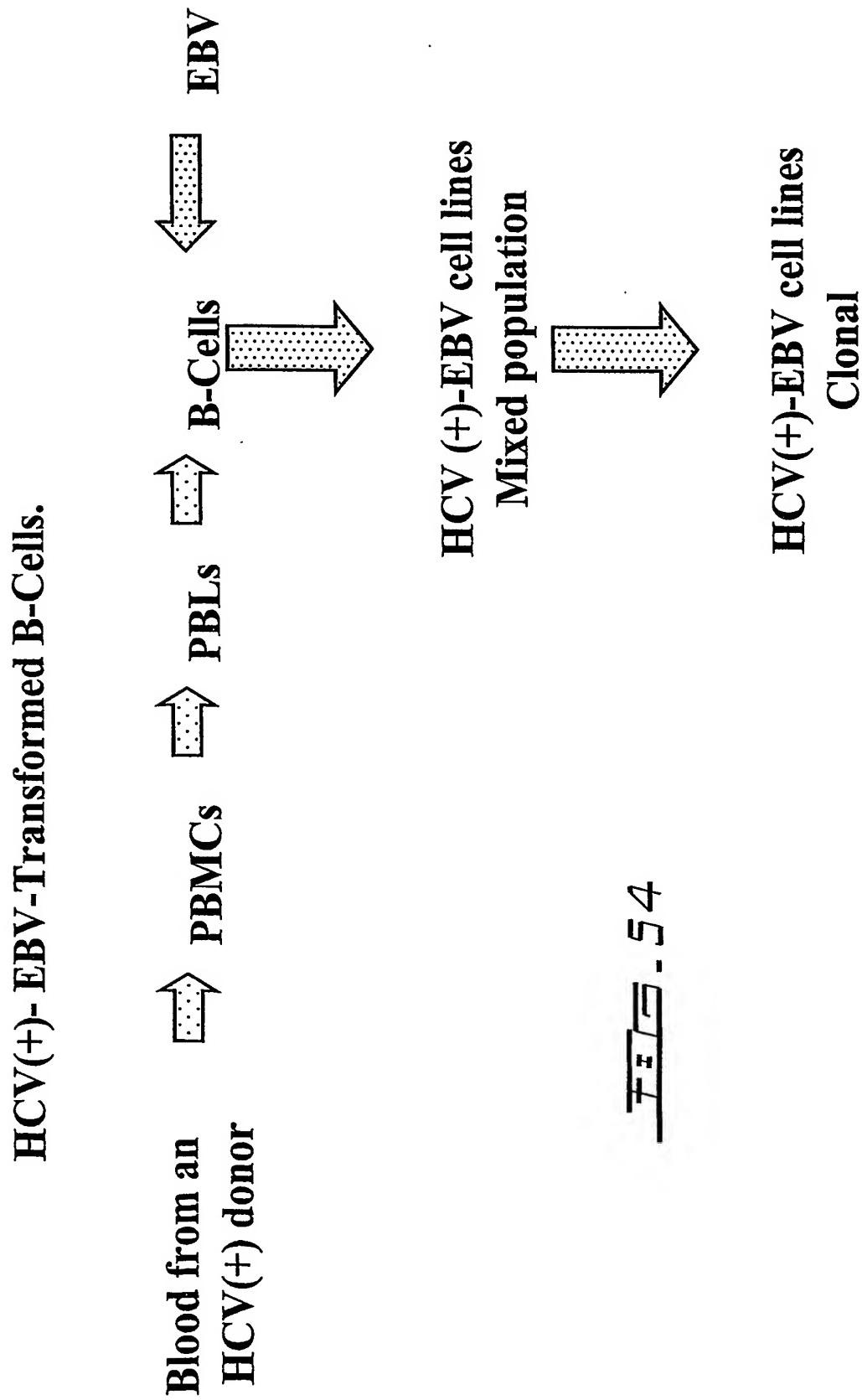
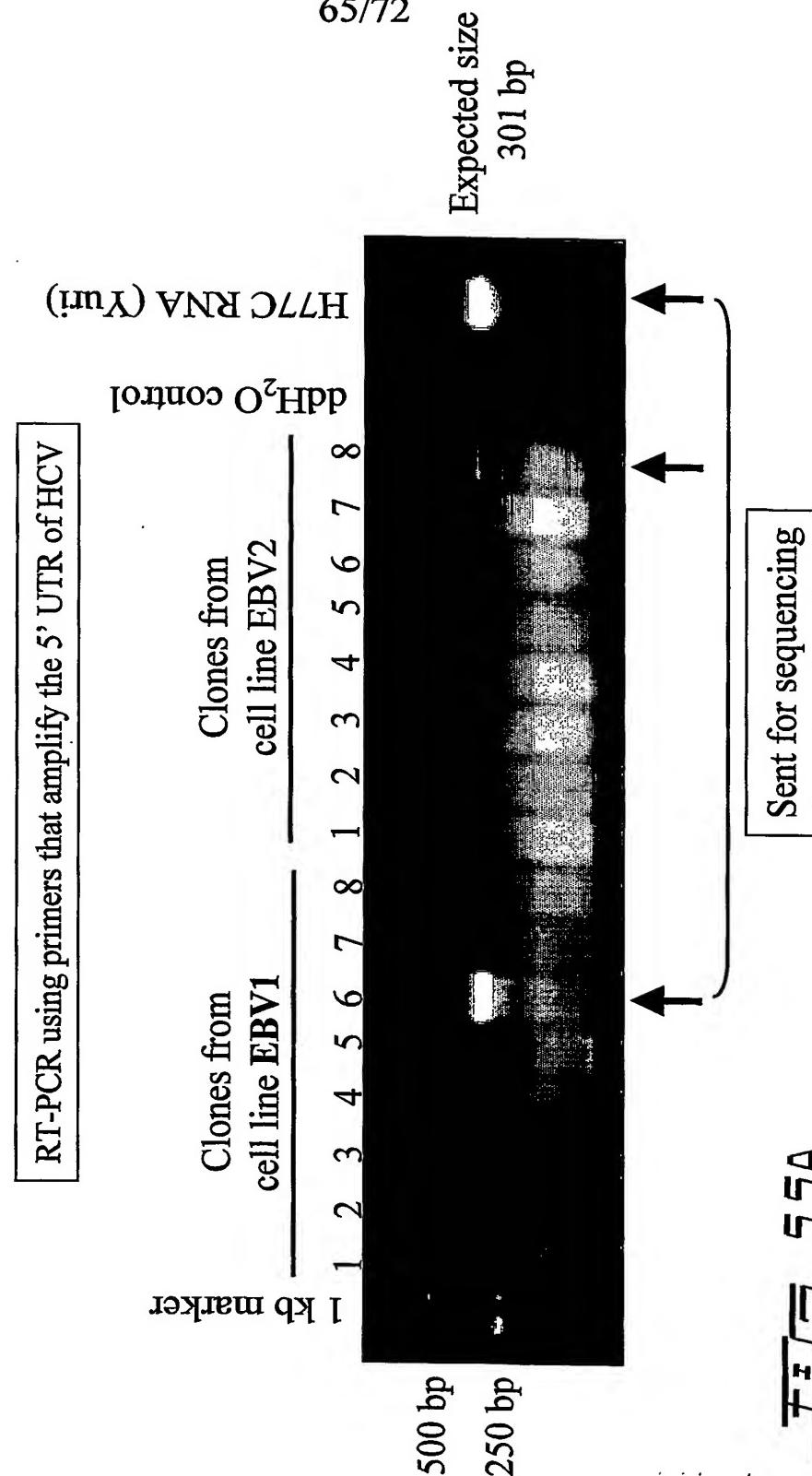


FIG. 53B

64/72



65/72



**Alignment: H77C (RT-PCR positive control) sequence (top)/  
EBV1 clone 6 sequence (bottom)**

CACTCCCTGTGAGGAACTA  
CACTCCCTGTGAGGAACTA  
TAGTATGAGTGTCCAGGACCCCC  
TAGTATGAGTGTCCAGGACCCCC  
  
TGCGGAACCGGGTGA  
TGCGGAACCGGGTGA  
ACCCGCTCACATGCC  
ACCCGCTCA -ATGCC  
TGTTGGGTCCGAAAGGC  
TGTTGGGTCCGAAAGGC  
  
G  
TGCGGAACCGGGTACACCCGGAAATTGCCAGGACGCC  
TGCGGAACCGGGTACACCCGGAAATTGCCAGGACGCC  
ACCCGCTCACATGCC  
ACCCGCTCA -ATGCC  
TGTTGGGTCCGAAAGGC  
TGTTGGGTCCGAAAGGC  
  
TTS - 55B

**Blue:** sequence from virus in the serum (MLL-005).

Alignment: H77C (RT-PCR positive control) sequence (top)/  
EBV2 clone 8 sequence (bottom).

CCAGGACCCCCCCTCCCGGGAGAGGCCATAGTGGTCTGCGGAACC  
CCAGGACCCCCCCTCCCGGGAGAGGCCATAGTGGTCTGCGGAACC

GGTGAGTACACCGGAATTGCCAGGACCGACCCGGTCTTCTTGG  
GGTGAGTACACCGGAATTGCCAGGACCGACCCGGTCTTCTTGG

ATAAAACCCGCTCAATGCCTGGAGATTGGCGTGCCTCCCCCAAG  
ATAAATCCCGCTCAATGCCTGGAGATTGGCGTGCCTCCCCCAAG

ACTGCTAGCCCCAGTAGTGTGGGTCTGGCGAAAGGCCCTGTGGTAC  
ACTGCTAGCCCCAGTAGTGTGGGTCTGGCGAAAGGCCCTGTGGTAC

TGCCTGATAGGGTCTGGCGAGTGCCTGGGGAGGTCTCGTAGAC  
TGCCTGATAGGGTCTGGCGAGTGCTCCGGGGAGGTCTCGTAGAC

CGTGCA  
CGTGCA

7375 - 55C

68/72

HT7C RNA (Y<sub>inf</sub>)ddH<sub>2</sub>O controlEBV1  
Clone 3  
(1.3\*)EBV1  
Clone 4  
(1.4)EBV1  
Clone 5  
(1.5)EBV1  
Clone 6  
(1.6)EBV1  
Clone 7  
(1.7)HT7C RNA (Y<sub>inf</sub>)ddH<sub>2</sub>O controlEBV1  
Clone 3  
(1.3\*)EBV1  
Clone 4  
(2.4\*)EBV1  
Clone 5  
(2.4\*)EBV1  
Clone 6  
(2.4\*)EBV1  
Clone 7  
(2.4\*)EBV1  
Clone 8  
(2.4\*)EBV1  
Clone 9  
(2.4\*)EBV1  
Clone 10  
(2.4\*)EBV1  
Clone 11  
(2.4\*)EBV1  
Clone 12  
(2.4\*)EBV1  
Clone 6  
(1.6)EBV1  
Clone 7  
(1.7)EBV1  
Clone 8  
(1.7)EBV1  
Clone 9  
(1.7)EBV1  
Clone 10  
(1.7)EBV1  
Clone 11  
(1.7)EBV1  
Clone 12  
(1.7)EBV9  
Clone 2  
(9.2\*)EBV9  
Clone 3  
(9.2\*)EBV9  
Clone 4  
(9.2\*)EBV9  
Clone 5  
(9.2\*)EBV9  
Clone 6  
(9.2\*)EBV9  
Clone 7  
(9.2\*)EBV9  
Clone 8  
(9.2\*)EBV9  
Clone 9  
(9.2\*)EBV9  
Clone 10  
(9.2\*)EBV9  
Clone 11  
(9.2\*)EBV9  
Clone 12  
(9.2\*)HT7C RNA  
ddH<sub>2</sub>O controlHT7C RNA  
ddH<sub>2</sub>O control

sequencing

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## Alignment of all 9.2 sequences

H77C	CACTCCCTGTGAGGAAC	ACTGTCTCACGCAGAAAGCGTCT
9.2 final seq	CACTCCCTGTGAGGAAC	ACTGTCTCACGCAGAAAGCGTCT
9.2a final seq	CACTCCCTGTGAGGAAC	ACTGTCTCACGCAGAAAGCGTCT
9.2b final seq	CACTCCCTGTGAGGAAC	ACTGTCTCACGCAGAAAGCGTCT
9.2c final seq	CACTCCCTGTGAGGAAC	ACTGTCTCACGCAGAAAGCGTCT
9.2d final seq	CACTCCCTGTGAGGAAC	ACTGTCTCACGCAGAAAGCGTCT

H77C	AGCCATGGCGTTAGTATGAGTGTGTCAGCAGGACCCCC
9.2 final seq	AGCCATGGCGTTAGTATGAGTGTGTCAGCAGGACCCCC
9.2a final seq	AGCCATGGCGTTAGTATGAGTGTGTCAGCAGGACCCCC
9.2b final seq	AGCCATGGCGTTAGTATGAGTGTGTC <u>A</u> CAGCCTCCAGGCC
9.2c final seq	AGCCATGGCGTTAGTATGAGTGTGTCAGCAGGACCCCC
9.2d final seq	AGCCATGGCGTTAGTATGAGTGTGTCAGCAGGACCCCC

H77C	CCTCCCCGGAGGCCATACTGGTCTGGAAACCGGTGAGTACAC
9.2 final seq	CCTCCCCGGAGGCCATACTGGTCTGGAAACCGGTGAGTACAC
9.2a final seq	CCTCCCCGGAGGCCATACTGGTCTGGAAACCGGTGAGTACAC
9.2b final seq	CCTCCCCGGAGGCCATACTGGTCTGGAAACCGGTGAGTACAC
9.2c final seq	CCTCCCCGGAGGCCATACTGGTCTGGAAACCGGTGAGTACAC
9.2d final seq	CCTCCCCGGAGGCCATACTGGTCTGGAAACCGGTGAGTACAC

F I T A - 57A

- = clone alone (not diluted with other cells)

a= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)

b= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)

c= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)

d= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

*Red= Variation with respect to clone 9.2*

70/72

## Alignment of all 9.2 sequences

H77C		CGGAATTGCCAGGACGGACCCGGTCCCTTCTTGGATAACC CGCT
9.2 final seq		CGGAATTGCCAGGACGGACCCGGTCCCTTCTTGGATAACC CGCT
9.2a final seq		CGGAATTGCCAGGACGGACCCGGTCCCTTCTTGGATA <u>T</u> ACC CGCT
<b>9.2b final seq</b>	<b>CGGAATTGCC<u>G</u>GA<u>A</u>GAC<u>T</u>GGGTCCCTTCTTGGATAAACCA<u>C</u>CT</b>	
9.2c final seq		CGGAATTGCCAGGACGGACCCGGTCCCTTCTTGGATAACC CGCT
9.2d final seq		CGGAATTGCCAGGACGGACCCGGTCCCTTCTTGGATA <u>T</u> AAATCC CGCT
H77C		CAATGCCCTGGAGATTGGCGTCCCCCGCAAGACTGCTAGGCC
9.2 final seq		CAATGCCCTGGAGATTGGCGTCCCCCGCAAGACTGCTAGGCC
9.2a final seq		CAATGCCCTGGAGATTGGCGTCCCCCGC <u>G</u> AGACTGCTAGGCC
<b>9.2b final seq</b>	<b>CTATGCCCGGC<u>C</u>ATTGGCGTCCCCCGCAAGACTGCTAGGCC</b>	
9.2c final seq		CAATGCCCTGGAGATTGGCGTCCCCCGCAAGACTGCTAGGCC
9.2d final seq		CAATGCCCTGGAGATTGGCGTCCCCCGC <u>G</u> AGACTGCTAGGCC

卷之二

**Alignment of all 9.2 sequences**

H77C	AGTAGTGTGGGTCTCGCGAAAGGCCTTGTGGTACTGCCCTGATAGG
9.2 final seq	AGTAGTGTGGGTCTCGCGAAAGGCCTTGTGGTACTGCCCTGATAGG
9.2a final seq	AGTAGTGTGGGTCTCGCGAAAGGCCTTGTGGTACTGCCCTGATAGG
<b>9.2b final seq</b>	<b>AGTAG<u>CG</u>TTGGGT<u>T</u>CGCGAAAGGCCTTGTGGTACTGCCCTGATAGG</b>
9.2c final seq	AGTAGTGTGGGTCTCGCGAAAGGCCTTGTGGTACTGCCCTGATAGG
9.2d final seq	AGTAGTGTGGGTCTCGCGAAAGGCCTTGTGGTACTGCCCTGATAGG
H77C	GTCGCTTGCGAGTGCCCCGGAGGGCTCCGTAGACCGCTGCA
9.2 final seq	GTCGCTTGCGAGTGCCCCGGAGGGCTCCGTAGACCGCTGCA
9.2a final seq	GTCGCTTGCGAGTGCCCCGGAGGGCTCCGTAGACCGCTGCA
<b>9.2b final seq</b>	<b>GTCGCTTGCGAGTGCCCCGGAGGGCTCCGTAGACCGCTGCA</b>
9.2c final seq	GTCGCTTGCGAGTGCCCCGGAGGGCTCCGTAGACCGCTGCA
9.2d final seq	GTCGCTTGCGAGTGCCCCGGAGGGCTCCGTAGACCGCTGCA

FIGURE - 57B (Cont.)

